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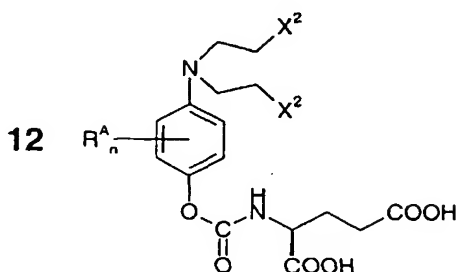
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(54) Title: **METHODS OF CHEMICAL SYNTHESIS OF PHENOLIC NITROGEN MUSTARD PRODRUGS**



(57) Abstract: This invention pertains to novel methods for the synthesis of certain nitrogen mustard prodrugs, such as N-[4-[N,N-bis(2-haloethylamino)-phenoxy]carbonyl]-L-glutamic acid: wherein: X₂ is a halo group, and is -F, -Cl, -Br, or -I; n is an integer from 0 to 4; and, each R_A is an aryl substituent. The methods comprise, at least, the steps of: glutamate conjugation (GC); silyloxy deprotection (SD); and, sulfonic esterification (SU). Certain preferred methods comprise the steps of: amine substitution (AS); silyloxy protection (SP); phenolic deprotection (PD); activation (AC); glutamate conjugation (GC); silyloxy deprotection (SD); sulfonic esterification (SU); halogenation (HL); glutamate deprotection (GD); and glutamic acid protection (GP).

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METHODS OF CHEMICAL SYNTHESIS OF PHENOLIC NITROGEN MUSTARD PRODRUGS

RELATED APPLICATION

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This application is related to (and where permitted by law, claims priority to) United Kingdom patent application number GB 0102239.1 filed 29 January 2001, the contents of which are incorporated herein by reference in their entirety.

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TECHNICAL FIELD

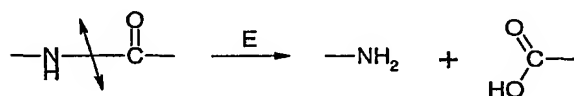
This invention pertains generally to the field of organic chemical synthesis, and more particularly to novel methods for the synthesis of certain nitrogen mustard prodrugs, such as N-{4-[N,N-bis(2-haloethylamino)-phenoxy]carbonyl}-L-glutamic acid, **12**.

15

BACKGROUND

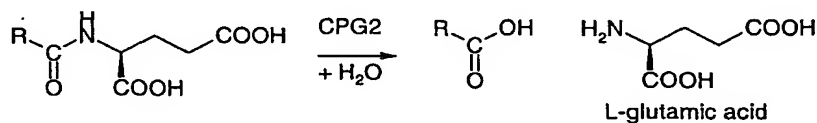
Peptidases are a class of enzymes (E) which act upon a substrate to cleave an amide linkage (-NH-C(=O)-) to give amino (-NH₂) and carboxylic acid (-C(=O)OH) products.

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One peptidase of particular interest is carboxypeptidase G2 (referred to herein as "CPG2"). A preferred substrate for this enzyme is an L-glutamic acid group, linked to an aromatic ring via an amidic, carbamic, or ureidic linkage.

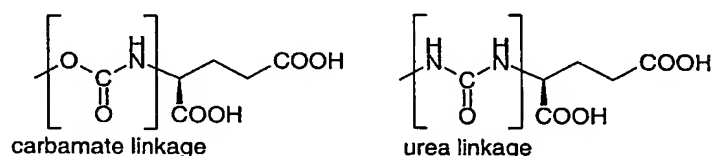
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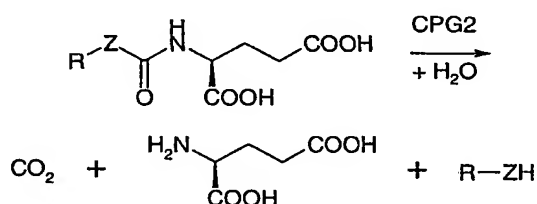
- 2 -

However, glutamic acid analogs are also acceptable substrates. For example, L-glutamic acid modified at the γ -carbon (e.g., with an amide, $-\text{CONH}_2$, instead of an acid, $-\text{COOH}$) also serves as a suitable substrate for CPG2.

- 5 CPG2 is also tolerant as to whether the amide group is naked, or is part of a larger linkage, for example, a carbamate or a urea linkage.



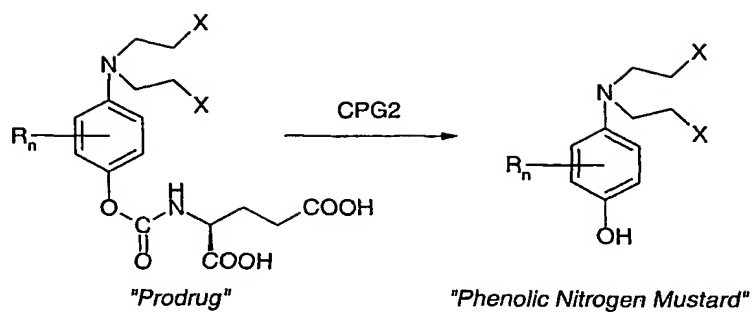
- For these compounds, CPG2 yields CO_2 , L-glutamic acid, and R-ZH , wherein
 10 when Z is $-\text{O}-$ (carbamates), R-ZH is a hydroxyl compound, R-OH , and when Z is $-\text{NH}-$ (ureas), R-ZH is an amino compound, R-NH_2 , where R is preferably an aromatic group.



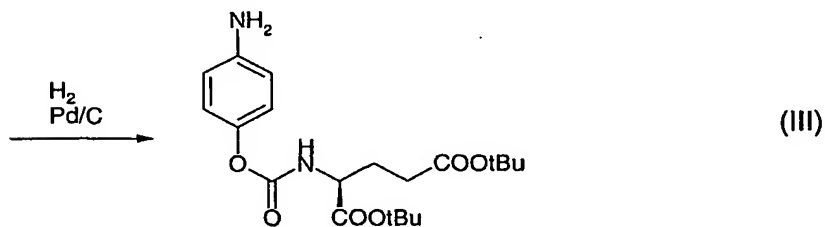
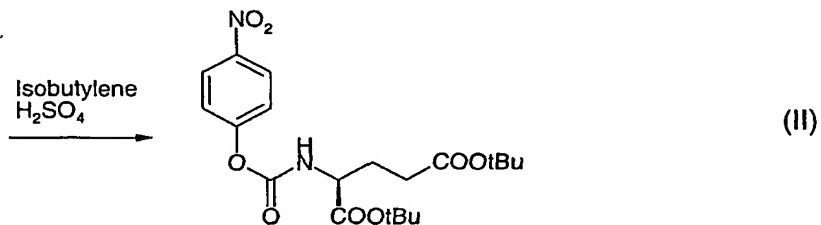
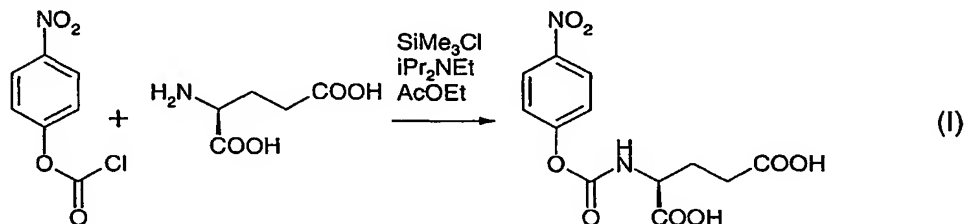
- 15 CPG2 and such substrates are useful in enzyme prodrug therapy (EPT) in which CPG2 may act upon a prodrug to yield an active compound. Examples of such therapies include antibody directed enzyme prodrug therapy (ADEPT), gene directed enzyme prodrug therapy (GDEPT), ligand directed enzyme prodrug therapy (LIDEPT), and bacteria directed enzyme prodrug therapy (BDEPT). See,
 20 for example, Burke, P. J., et al., 1994; Springer, C. J., et al., 1995; Blakey, D. C., et al., 1996; Springer, C. J., et al., 1996; Emery, S. C., et al., 1998.

- In one class of therapy, CPG2 acts upon a prodrug to yield a phenolic nitrogen mustard compound which is useful, for example, in the treatment of a proliferative
 25 condition, for example, cancer.

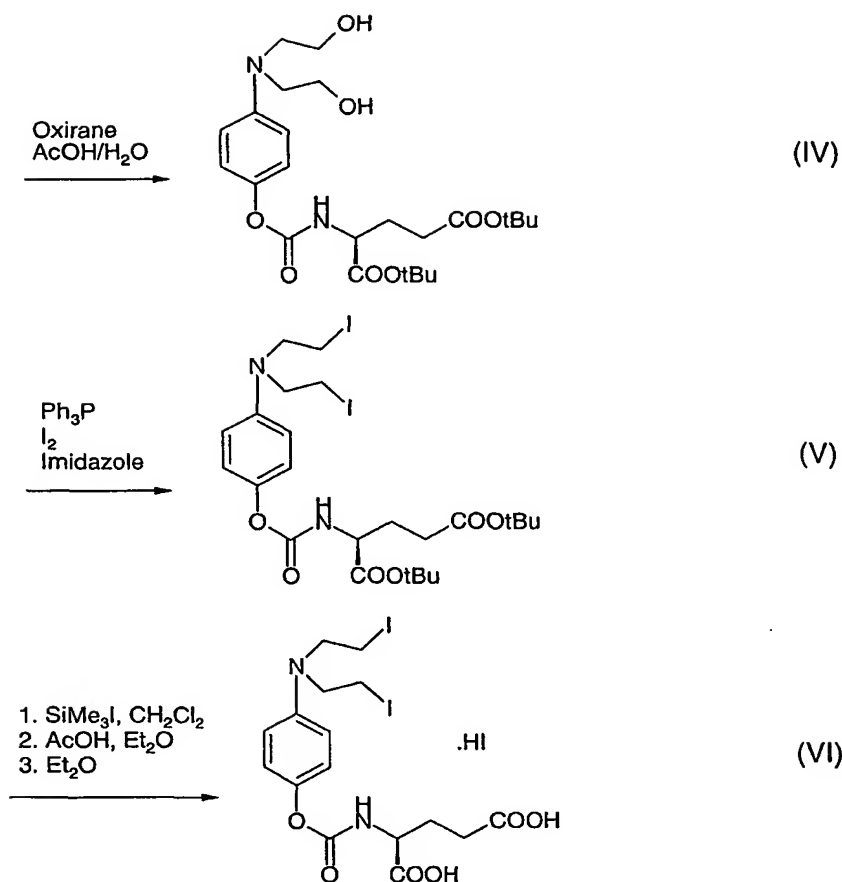
- 3 -



One method for the synthesis of such prodrug compounds is described in Burke, P. J., et al., 1994. An improved method was subsequently described in Heaton, D. W., et al., 1996 (see Figure 2 therein) and is shown below:



- 4 -



This method suffers numerous disadvantages including several difficult and low yield reaction steps, such as:

- 5 (a) Step (I): 4-nitrophenyl chloroformate coupling (77% with respect to glutamic acid),
 - (b) Step (III): reduction of nitroderivative to amine (58%); and,
 - (c) Step (IV): hydroxyethylation, which is generally a difficult and low yield reaction (49%).
- 10 The present invention relates to new methods for the synthesis of such prodrug compounds, such as N-{4-[N,N-bis(2-haloethylamino)-phenoxy-carbonyl]-L-glutamic acid. These new methods have one or more of the following advantages:

- 5 -

(1) higher yield of the desired prodrug with respect to the reagent, glutamic acid, than has been obtained in previous methods.

(2) higher yield of the glutamate conjugation reaction with respect to the precursors (e.g., **5** + **15** to give **6**; **5A** + **15A** to give **6A**) than has been obtained
5 in the corresponding conjugation reaction of previous methods (e.g., Step (I) above).

(3) amine substitution (e.g., **1** to **2**; **1A** to **2A**) is performed earlier and with a cheaper reagent (e.g., 4-benzyloxyaniline, **1A**) than in previous methods (e.g., the more expensive and advanced intermediate di-t-butyl, N-(4-amino-
10 phenoxy carbonyl)-L-glutamate, in Step (IV) above).

(4) higher yield of amine substitution (e.g., **1** to **2**; **1A** to **2A**) than has been obtained in the corresponding step in previous methods (e.g., Step (IV) above).

(5) simplified work-up of the product of amine substitution (e.g., **1** to **2**; **1A** to **2A**) with respect to that in previous methods (e.g., Step (IV) above).

(6) introduction of the glutamic acid/glutamate residue at a later step (e.g.,
15 **5** + **15** to give **6**; **5A** + **15A** to give **6A**) than in previous methods (e.g., Step (I) above), and thereby improving the economy of the overall process, in regard to this reagent.

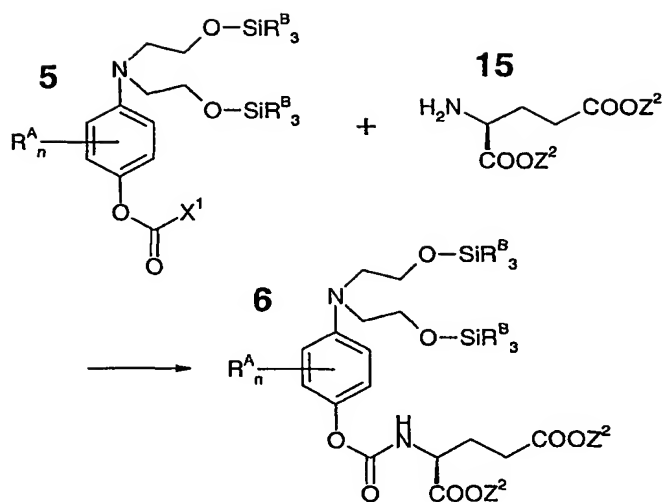
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SUMMARY OF INVENTION

One aspect of the invention pertains to a method of synthesis comprising the steps of, in order:

glutamate conjugation (GC), in which a
25 4-[N,N-bis(2-silyloxyethyl)amino]phenyl haloformate, activated carbonate, or activated carbamate **5**, reacts with a glutamate, **15**, to give an N-{4-[N,N-bis(2-silyloxyethyl) amino]phenoxy carbonyl}-L-glutamate, **6**:

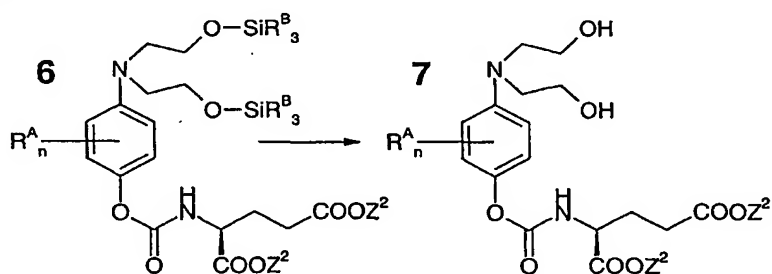
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silyloxy deprotection (SD), in which said

N-{4-[N,N-bis(2-silyloxyethyl)amino]phenoxy-carbonyl}-L-glutamate, 6, is converted to an N-{4-[N,N-bis(2-hydroxyethyl)amino]phenoxy-carbonyl}-

5 L-glutamate, 7:

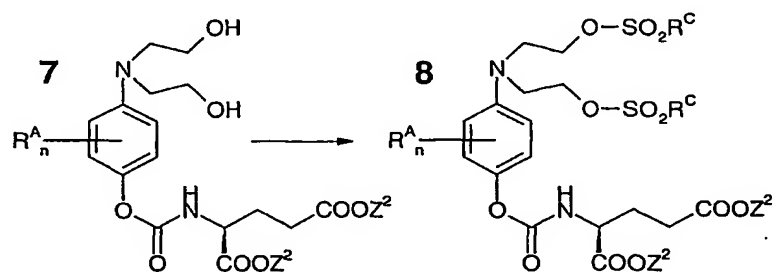


sulfonic esterification (SE), in which said

N-{4-[N,N-bis(2-hydroxyethyl)amino]phenoxy-carbonyl}-L-glutamate, 7, is

converted to an N-{4-[N,N-bis(2-sulfonyloxyethyl)amino]phenoxy-carbonyl}-

10 L-glutamate, 8:



wherein:

- 7 -

X^1 is a leaving group;

n is an integer from 0 to 4;

each R^A is independently an aryl substituent;

each R^B is a silyl substituent, and is independently C_{1-7} alkyl,

5 C_{3-20} heterocyclyl, or C_{5-20} aryl, and is optionally substituted;

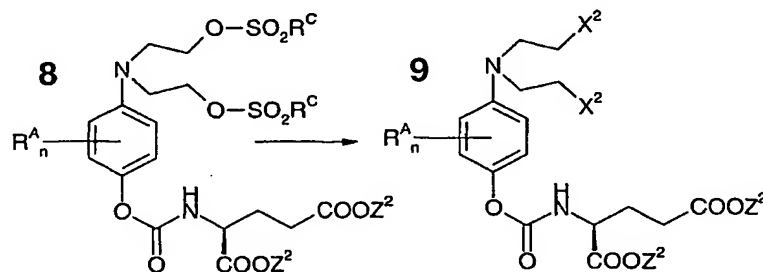
each R^C is a sulfonic substituent, and is independently C_{1-7} alkyl,

C_{3-20} heterocyclyl, or C_{5-20} aryl, and is optionally substituted; and,

each Z^2 is independently a carboxylic acid protecting group.

10 In one embodiment, the method further comprises, following the step of sulfonic esterification (SE), the additional step of:

halogenation (HL), in which said N-{4-[N,N-bis(2-sulfonyloxyethyl)amino]phenoxy}carbonyl-L-glutamate, **8**, is converted to an N-{4-[N,N-bis(2-haloethyl)amino]phenoxy}carbonyl-L-glutamate, **9**:



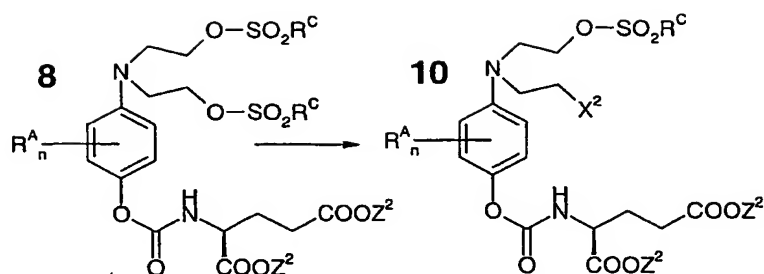
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wherein each X^2 is independently -F, -Cl, -Br, -I.

In one embodiment, the method further comprises, following the step of sulfonic esterification (SE), the additional step of:

20 halogenation (HL), in which said N-{4-[N,N-bis(2-sulfonyloxyethyl)amino]phenoxy}carbonyl-L-glutamate, **8**, is converted to an N-{4-[N-(2-sulfonyloxyethyl),N-(2-haloethyl)amino]phenoxy}carbonyl-L-glutamate, **10**:

- 8 -

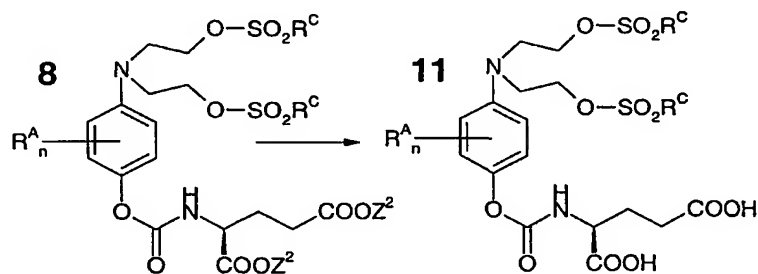


wherein each X^2 is independently -F, -Cl, -Br, -I.

In one embodiment, the method further comprises, following the step of sulfonic esterification (SE), the additional step of:

glutamate deprotection (GD), in which said

N-(4-[N,N-bis(2-sulfonyloxyethyl)amino]phenoxy)carbonyl-L-glutamate, **8**, is converted to an N-(4-[N,N-bis(2-sulfonyloxyethyl)amino]phenoxy)carbonyl-L-glutamic acid, **11**:



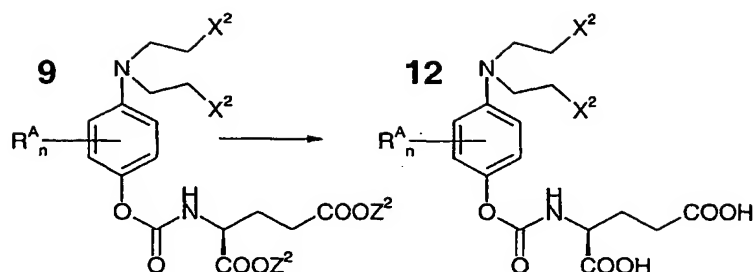
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In one embodiment, the method further comprises, following the step of halogenation (HL), the additional step of:

glutamate deprotection (GD), in which said

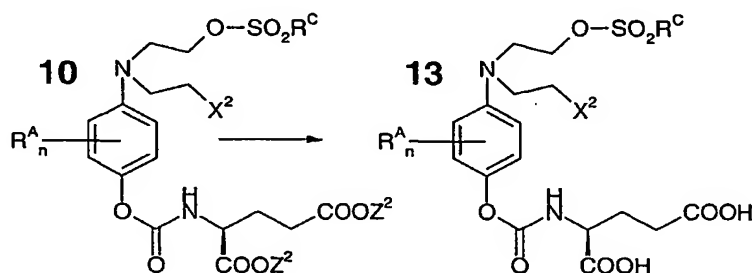
N-(4-[N,N-bis(2-haloethyl)amino]phenoxy)carbonyl-L-glutamate, **9**, is converted to an N-(4-[N,N-bis(2-haloethyl)amino]phenoxy)carbonyl-L-glutamic acid, **12**:

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In one embodiment, the method further comprises, following the step of halogenation (HL), the additional step of:

- 5 glutamate deprotection (GD), in which said N-{4-[N-(2-sulfonyloxy ethyl),N-(2-haloethyl)amino] phenoxy carbonyl}-L-glutamate, **10**, is converted to an N-{4-[N-(2-sulfonyloxyethyl),N-(2-haloethyl)amino] phenoxy carbonyl}-L-glutamic acid, **13**:

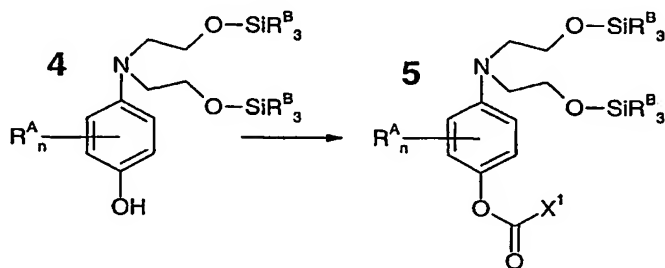


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In one embodiment, the method further comprises, preceding the step of glutamate conjugation (GC), the additional step of:

activation (AC), in which a 4-[N,N-bis(2-silyloxyethyl)amino]phenol, **4**, is converted to said 4-[N,N-bis(2-silyloxyethyl)amino]phenyl haloformate, activated

- 15 carbonate, or activated carbamate **5**:

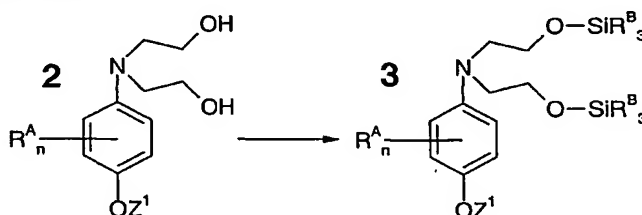


In one embodiment, the method further comprises, preceding the step of

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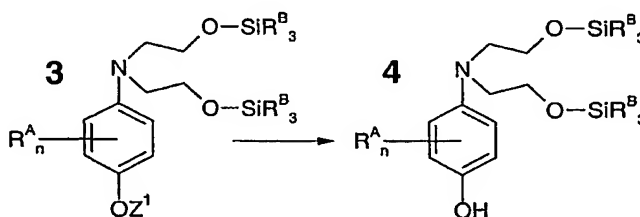
activation (AC), the additional steps of:

silyloxy protection (SP), in which a 4-(protected hydroxy)-N,N-bis(2-hydroxyethyl)aniline, **2**, is converted to a 4-(protected hydroxy)-N,N-bis(2-silyloxyethyl) aniline, **3**:



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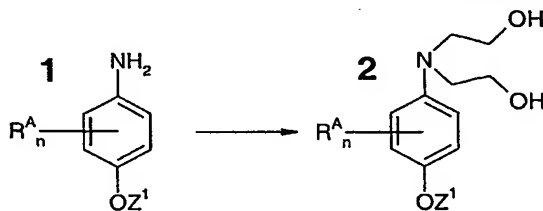
phenolic deprotection (PD), in which said 4-(protected hydroxy)-N,N-bis(2-silyloxyethyl)aniline, **3**, is converted to said 4-[N,N-bis(2-silyloxyethyl)amino] phenol, **4**:



10 wherein Z^1 is a phenol protecting group.

In one embodiment, the method further comprises, preceding the step of silyloxy protection (SP), the additional step of:

15 amine substitution (AS), in which a 4-(protected hydroxy)-aniline, **1**, is converted to said 4-(protected hydroxy)-N,N-bis(2-hydroxyethyl)aniline, **2**:



In one embodiment, the method further comprises, at any time preceding the step of glutamate conjugation (GC), the parallel step of:

20 glutamate protection (GP), in which L-glutamic acid, **14**, is converted to

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said L-glutamate, **15**:



In one embodiment, said glutamate conjugation (GC) step is achieved by reaction
 5 in the presence of a base. In one embodiment, said glutamate conjugation (GC) step is achieved by reaction in the presence of the base triethylamine.

In one embodiment, said silyloxy deprotection (SD) step is achieved by reaction
 10 with triethylamine trihydrofluoride ($\text{NEt}_3 \cdot 3\text{HF}$).

In one embodiment, said sulfonic esterification (SE) step is achieved by reaction
 with a sulfonic anhydride, $(\text{R}^{\text{C}}\text{SO}_2)_2\text{O}$. In one embodiment, said sulfonic
 esterification (SE) step is achieved by reaction with a sulfonic anhydride,
 $(\text{R}^{\text{C}}\text{SO}_2)_2\text{O}$, in the presence of a base. In one embodiment, said sulfonic
 15 esterification (SE) step is achieved by reaction with a sulfonic anhydride,
 $(\text{R}^{\text{C}}\text{SO}_2)_2\text{O}$, in the presence of one or both of triethylamine and
 4-dimethylaminopyridine. In one embodiment, said sulfonic anhydride is
 methanesulfonic anhydride, $(\text{MeSO}_2)_2\text{O}$.

20 In one embodiment, said halogenation (HL) step is achieved by reaction with an
 alkaline halide. In one embodiment, said alkaline halide is sodium iodide.

In one embodiment, said glutamate deprotection (GD) step is achieved by
 reaction with acid. In one embodiment, said glutamate deprotection (GD) step is
 25 achieved by reaction with trifluoroacetic acid.

In one embodiment, said activation (AC) step is achieved by reaction with a
 carbonyl halide, $\text{X}^1\text{C}(=\text{O})\text{X}^1$. In one embodiment, said activation (AC) step is
 achieved by reaction with a carbonyl halide, $\text{X}^1\text{C}(=\text{O})\text{X}^1$ in the presence of a
 30 base. In one embodiment, said activation (AC) step is achieved by reaction with

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a carbonyl halide, $X^1C(=O)X^1$ in the presence of the base triethylamine. In one embodiment, said carbonyl halide is phosgene, $ClC(=O)Cl$.

In one embodiment, said silyloxy protection (SP) step is achieved by reaction with
5 the corresponding halosilane, SiR^B_3X . In one embodiment, said silyloxy
protection (SP) step is achieved by reaction with the corresponding chlorosilane,
 SiR^B_3Cl . In one embodiment, said silyloxy protection (SP) step is achieved by
reaction with the corresponding halosilane, SiR^B_3X , in the presence of a base. In
one embodiment, said silyloxy protection (SP) step is achieved by reaction with
10 the corresponding halosilane, SiR^B_3X , in the presence of the base imidazole.

In one embodiment, said phenolic deprotection (PD) step is achieved by
treatment with hydrogen gas and a palladium/carbon catalyst.

15 In one embodiment, said amine substitution (AS) step is achieved by treatment
with ethylene oxide. In one embodiment, said amine substitution (AS) step is
achieved by treatment with ethylene oxide in acetic acid, in the presence of a
base. In one embodiment, said amine substitution (AS) step is achieved by
treatment with ethylene oxide in acetic acid, in the presence of the base
20 triethylamine.

In one embodiment, said glutamate protection (GP) step is achieved by reaction
with isobutylene in the presence of sulfuric acid.

25 In one embodiment, X^1 is -F, -Cl, -Br, p-nitrophenoxy, pentafluorophenoxy,
succinimidyloxy, or imidazolyl. In one embodiment, X^1 is -F, -Cl, or -Br. In one
embodiment, X^1 is -Cl. In one embodiment, X^1 is p-nitrophenoxy,
pentafluorophenoxy, succinimidyloxy. In one embodiment, X^1 is imidazolyl.

30 In one embodiment, each of R^{A2} , R^{A3} , R^{A5} , and R^{A6} is independently -H or an aryl
substituent selected from: halo; ether; thioether; acyl; ester; amido; disubstituted
amino; cyano; nitro; and, C_{1-7} alkyl.

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In one embodiment, each of R^{A2} , R^{A3} , R^{A5} , and R^{A6} is independently -H, -F, -Cl, -CONH₂, -CN, or -Me.

5 In one embodiment, each of R^{A2} , R^{A3} , R^{A5} , and R^{A6} is independently -H or -Me.

In one embodiment, n is 0 or each R^A is -H.

10 In one embodiment, each R^B is independently -Me, -Et, -nPr, -iPr, -nBu, -sBu, -iBu, -tBu, or -Ph. In one embodiment, each $-SiR^B_3$ group is independently $-Si(Me)_3$, $-Si(Et)_3$, $-Si(iPr)_3$, $-Si(tBu)(Me)_2$, or $-Si(Ph)_2(tBu)$. In one embodiment, each $-SiR^B_3$ groups is $-Si(tBu)(Me)_2$.

In one embodiment, each R^C is C₁₋₇alkyl or C₅₋₂₀aryl, and is optionally substituted.

15 In one embodiment, each R^C is -Me. In one embodiment, each R^C is phenyl or substituted phenyl.

In one embodiment, each Z^2 is independently t-butyl, allyl, or benzyl. In one embodiment, each Z^2 is t-butyl.

20

In one embodiment, X^2 is -Cl, -Br, or -I. In one embodiment, X^2 is -Cl. In one embodiment, X^2 is -I.

In one embodiment, Z^1 is benzyl or allyl. In one embodiment, Z^1 is benzyl.

25

Another aspect of the present invention pertains to compounds obtainable by a method as described herein, or a method comprising a method as described herein. In one embodiment, the present invention pertains to a compound of the formula **11**, **12**, or **13** obtainable by a method comprising a method as described

30 herein.

- 14 -

Another aspect of the present invention pertains to compounds obtained by a method as described herein, or a method comprising a method as described herein. In one embodiment, the present invention pertains to a compound of the formula **11**, **12**, or **13** obtained by a method comprising a method as described
5 herein.

Another aspect of the present invention pertains to novel intermediates, as described herein, which are suitable for use in the methods of synthesis described herein. Examples of such intermediates include **3** (e.g., **3A**),
10 **4** (e.g., **4A**), **5** (e.g., **5A**), and **6** (e.g., **6A**).

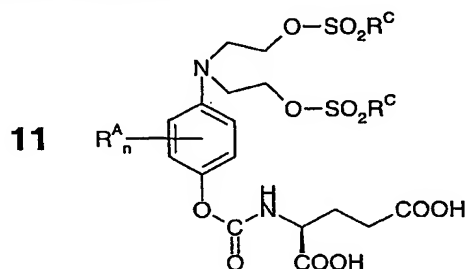
Another aspect of the present invention pertains to the use of such novel intermediates, as described herein, in the methods of synthesis described herein.

15 As will be appreciated by one of skill in the art, features and preferred embodiments of one aspect of the invention will also pertain to other aspect of the invention.

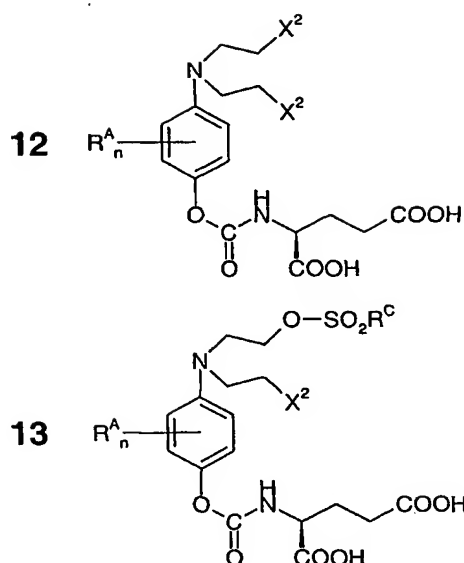
DETAILED DESCRIPTION OF THE INVENTION

20

The present invention pertains to new methods for the synthesis of compounds of the following formulae, and/or intermediates from which compounds of the following formulae may be prepared:



- 15 -



wherein:

- each X^2 is independently -F, -Cl, -Br, -I;
- n is an integer from 0 to 4;
- each R^A is an aryl substituent; and,
- each R^C is a sulfonic substituent, and is independently C_{1-7} alkyl, C_{3-20} heterocyclyl, or C_{5-20} aryl, and is optionally substituted.
- (1) In one embodiment, the method comprises the steps of, in order:
- glutamate conjugation (GC);
 - silyloxy deprotection (SD); and,
 - sulfonic esterification (SU).
- (2) In one embodiment, the method comprises the steps of, in order:
- glutamate conjugation (GC);
 - silyloxy deprotection (SD);
 - sulfonic esterification (SU); and,
 - halogenation (HL).

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method comprises the steps of, in order:

glutamate conjugation (GC);

silyloxy deprotection (SD);

sulfonic esterification (SU);

- 5 (3) In one embodiment, the and,
glutamate deprotection (GD).

(4) In one embodiment, the method comprises the steps of, in order:

glutamate conjugation (GC);

- 10 silyloxy deprotection (SD);

sulfonic esterification (SU);

halogenation (HL); and,

glutamate deprotection (GD).

- 15 (5) In one embodiment, the method comprises the steps of, in order:

activation (AC);

glutamate conjugation (GC);

silyloxy deprotection (SD); and,

sulfonic esterification (SU).

20

(6) In one embodiment, the method comprises the steps of, in order:

activation (AC);

glutamate conjugation (GC);

silyloxy deprotection (SD);

- 25 sulfonic esterification (SU); and,

halogenation (HL).

- 17 -

(7) In one embodiment, the method comprises the steps of, in order:

- activation (AC);
- glutamate conjugation (GC);
- silyloxy deprotection (SD);
- 5 sulfonic esterification (SU); and,
- glutamate deprotection (GD).

(8) In one embodiment, the method comprises the steps of, in order:

- activation (AC);
- 10 glutamate conjugation (GC);
- silyloxy deprotection (SD);
- sulfonic esterification (SU);
- halogenation (HL); and,
- glutamate deprotection (GD).

15

(9) In one embodiment, the method comprises the steps of, in order:

- silyloxy protection (SP);
- phenolic deprotection (PD);
- activation (AC);
- 20 glutamate conjugation (GC);
- silyloxy deprotection (SD); and,
- sulfonic esterification (SU).

(10) In one embodiment, the method comprises the steps of, in order:

- 25 silyloxy protection (SP);
- phenolic deprotection (PD);
- activation (AC);
- glutamate conjugation (GC);
- silyloxy deprotection (SD);
- 30 sulfonic esterification (SU); and,
- halogenation (HL).

- 18 -

- (11) In one embodiment, the method comprises the steps of, in order:
silyloxy protection (SP);
phenolic deprotection (PD);
activation (AC);
5 glutamate conjugation (GC);
silyloxy deprotection (SD);
sulfonic esterification (SU); and,
glutamate deprotection (GD).
- 10 (12) In one embodiment, the method comprises the steps of, in order:
silyloxy protection (SP);
phenolic deprotection (PD);
activation (AC);
glutamate conjugation (GC);
15 silyloxy deprotection (SD);
sulfonic esterification (SU);
halogenation (HL); and,
glutamate deprotection (GD).
- 20 (13) In one embodiment, the method comprises the steps of, in order:
amine substitution (AS);
silyloxy protection (SP);
phenolic deprotection (PD);
activation (AC);
25 glutamate conjugation (GC);
silyloxy deprotection (SD); and,
sulfonic esterification (SU).
- (14) In one embodiment, the method comprises the steps of, in order:
30 amine substitution (AS);
silyloxy protection (SP);
phenolic deprotection (PD);

- 19 -

activation (AC);
glutamate conjugation (GC);
silyloxy deprotection (SD);
sulfonic esterification (SU); and,
5 halogenation (HL).

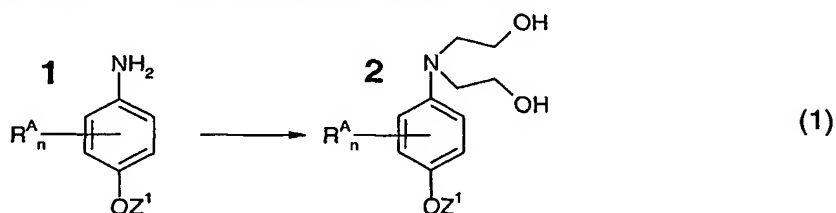
(15) In one embodiment, the method comprises the steps of, in order:
amine substitution (AS);
silyloxy protection (SP);
10 phenolic deprotection (PD);
activation (AC);
glutamate conjugation (GC);
silyloxy deprotection (SD);
sulfonic esterification (SU); and,
15 glutamate deprotection (GD).

(16) In one embodiment, the method comprises the steps of, in order:
amine substitution (AS);
silyloxy protection (SP);
20 phenolic deprotection (PD);
activation (AC);
glutamate conjugation (GC);
silyloxy deprotection (SD);
sulfonic esterification (SU);
25 halogenation (HL); and,
glutamate deprotection (GD).

(17) In one embodiment, the method additionally comprises the parallel step of
glutamate protection (GP), at a time before glutamate conjugation (GC).
30

Amine Substitution (AS)

In this step, a 4-(protected hydroxy)-aniline, **1**, is converted to a 4-(protected hydroxy)-N,N-bis(2-hydroxyethyl)aniline, **2**.



5

The group $-OZ^1$ is a protected hydroxy group. The group, $-Z^1$ is a suitable phenol protecting group. For a review of phenol protecting groups, see, for example, Greene et al., 1999a. In one preferred embodiment, the group, $-Z^1$, is allyl or benzyl. In one preferred embodiment, the group, $-Z^1$, is benzyl.

10

In one preferred embodiment, n is 0.

In one preferred embodiment, n is 0 or 1.

In one preferred embodiment, n is 0, 1, or 2.

In one preferred embodiment, n is 0, 1, 2, or 3.

15 In one preferred embodiment, n is 0, 1, 2, 3, or 4.

In one preferred embodiment, n is 1.

In one preferred embodiment, n is 1 or 2.

In one preferred embodiment, n is 1, 2, or 3.

In one preferred embodiment, n is 1, 2, 3, or 4.

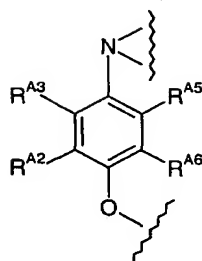
20 In one preferred embodiment, n is 2.

In one preferred embodiment, n is 3.

In one preferred embodiment, n is 4.

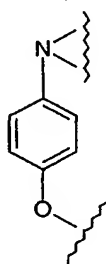
In one preferred embodiment, the substituted phenylene group has the following
25 formula:

- 21 -



wherein each of R^{A2} , R^{A3} , R^{A5} , and R^{A6} is independently -H or an aryl substituent.

In one preferred embodiment, each of R^{A2} , R^{A3} , R^{A5} , and R^{A6} is -H.



5

In one preferred embodiment, each of R^{A2} , R^{A3} , R^{A5} , and R^{A6} is independently -H or an aryl substituent selected from: halo; ether (e.g., C_{1-7} alkoxy); acyl (e.g., C_{1-7} alkylacyl, C_{5-20} arylacyl); ester; acyloxy; amido; acylamido; thioamido; tetrazolyl; disubstituted amino; nitro; azido; cyano; cyanato; thiocyno; thioether (e.g., C_{1-7} alkylthio); sulfonate; sulfone; sulfonyloxy; sulfinyloxy; sulfonamino; sulfinamino; sulfamyl; sulfonamido; C_{1-7} alkyl (including, e.g., C_{1-7} haloalkyl, C_{5-20} aryl- C_{1-7} alkyl); C_{3-20} heterocyclyl; or C_{5-20} aryl (including, e.g., C_{5-20} carboaryl, C_{5-20} heteroaryl, C_{1-7} alkyl- C_{5-20} aryl and C_{5-20} haloaryl).

15

In one preferred embodiment, each of R^{A2} , R^{A3} , R^{A5} , and R^{A6} is independently -H or an aryl substituent selected from:

-F, -Cl, -Br, and -I;

-OMe, -OEt, -O(tBu), and -OCH₂Ph;

20 -SMe, -SEt, -S(tBu), and -SCH₂Ph;

-C(=O)Me, -C(=O)Et, -C(=O)(tBu), and -C(=O)Ph;

-C(=O)OMe, -C(=O)OEt, and -C(=O)O(tBu);

- 22 -

- C(=O)NH₂, -C(=O)NHMe, -C(=O)NMe₂, and -C(=O)NH₂Et;
 -NHC(=O)Me, -NHC(=O)Et, -NHC(=O)Ph, succinimidyl, and maleimidyl;
 -CN;
 -NMe₂, -NEt₂, -N(iPr)₂, -N(nPr)₂, -N(nBu)₂, and -N(tBu)₂;
 5 -NO₂;
 -NMe₂, -NEt₂, -N(iPr)₂, -N(nPr)₂, -N(nBu)₂, and -N(tBu)₂;
 -Me, -Et, -nPr, -iPr, -nBu, -tBu;
 -CF₃, -CHF₂, -CH₂F, -CCl₃, -CBr₃, -CH₂CH₂F, -CH₂CHF₂, and -CH₂CF₃;
 -OCF₃, -OCHF₂, -OCH₂F, -OCCl₃, -OCBr₃, -OCH₂CH₂F, -OCH₂CHF₂, and
 10 -OCH₂CF₃;
 -CH₂CH₂NMe₂; and,
 optionally substituted phenyl.

- In one preferred embodiment, each of R^{A2}, R^{A3}, R^{A5}, and R^{A6} is independently -H
 15 or an aryl substituent selected from: halo; ether (e.g., C₁₋₇alkoxy); thioether; acyl;
 ester; amido; disubstituted amino; cyano; nitro; and, C₁₋₇alkyl (including, e.g.,
 C₁₋₇haloalkyl, C₅₋₂₀aryl-C₁₋₇alkyl).

- In one preferred embodiment, each of R^{A2}, R^{A3}, R^{A5}, and R^{A6} is independently -H
 20 or an aryl substituent selected from:
 -F, -Cl, -Br, and -I;
 -OMe, -OEt, -O(tBu), and -OCH₂Ph;
 -SMe, -SEt, -S(tBu), and -SCH₂Ph;
 -C(=O)Me, -C(=O)Et, -C(=O)(tBu), and -C(=O)Ph;
 25 -C(=O)OMe, -C(=O)OEt, and -C(=O)O(tBu);
 -C(=O)NH₂, -C(=O)NHMe, -C(=O)NMe₂, and -C(=O)NH₂Et;
 -NMe₂, -NEt₂, -N(iPr)₂, -N(nPr)₂, -N(nBu)₂, and -N(tBu)₂;
 -CN;
 -NO₂;
 30 -Me, -Et, -nPr, -iPr, -nBu, -tBu;
 -CF₃, -CHF₂, -CH₂F, -CCl₃, -CBr₃, -CH₂CH₂F, -CH₂CHF₂, and -CH₂CF₃; and
 -CH₂CH₂NMe₂.

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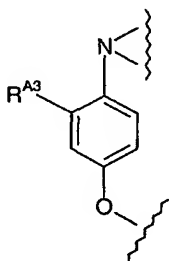
In one preferred embodiment, each of R^{A2} , R^{A3} , R^{A5} , and R^{A6} is independently -H or an aryl substituent selected from: -F, -Cl, -Br, -I, -OMe, -OEt, -SMe, -SEt, -C(=O)Me, -C(=O)OMe, -CONH₂, -CONHMe, -NMe₂, -NEt₂, -N(nPr)₂, -N(iPr)₂,
 5 -CN, -NO₂, -Me, -Et, -CF₃, -OCF₃, and -Ph.

In one preferred embodiment, each of R^{A2} , R^{A3} , R^{A5} , and R^{A6} is independently -H or an aryl substituent selected from:
 -F, -Cl, -CONH₂, -CN, and -Me.

10

In one preferred embodiment, each of R^{A2} , R^{A3} , R^{A5} , and R^{A6} is independently -H or -Me.

In one preferred embodiment, each of R^{A2} , R^{A5} , and R^{A6} is -H, and R^{A3} is as
 15 defined above.

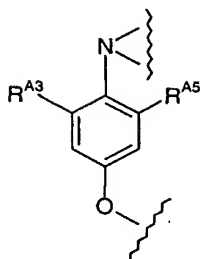


In one preferred embodiment, each of R^{A2} , R^{A5} , and R^{A6} is -H, and R^{A3} is -H, -F, -Cl, -CONH₂, -CN, or -Me.

20 In one preferred embodiment, each of R^{A2} , R^{A5} , and R^{A6} is -H, and R^{A3} is -H or -Me.

In one preferred embodiment, each of R^{A2} and R^{A6} is -H, and each of R^{A3} and R^{A5} is independently as defined above.

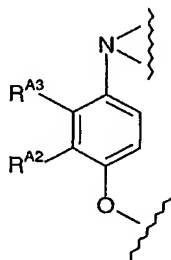
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In one preferred embodiment, each of R^{A2} and R^{A6} is -H, and each of R^{A3} and R^{A5} is independently -H, -F, -Cl, -CONH₂, -CN, or -Me.

- 5 In one preferred embodiment, each of R^{A2} and R^{A6} is -H, and each of R^{A3} and R^{A5} is independently -H or -Me.

In one preferred embodiment, each of R^{A5} and R^{A6} is -H, and each of R^{A2} and R^{A3} is independently as defined above.



10

In one preferred embodiment, each of R^{A5} and R^{A6} is -H, and each of R^{A2} and R^{A3} is independently -H, -F, -Cl, -CONH₂, -CN, or -Me.

- 15 In one preferred embodiment, each of R^{A5} and R^{A6} is -H, and each of R^{A2} and R^{A3} is independently -H or -Me.

The conversion of the amino group, -NH₂, to a bis(2-hydroxyethyl)amino group, -N(CH₂CH₂OH)₂, may be achieved using well known methods. See, for example, Everett et al., 1949.

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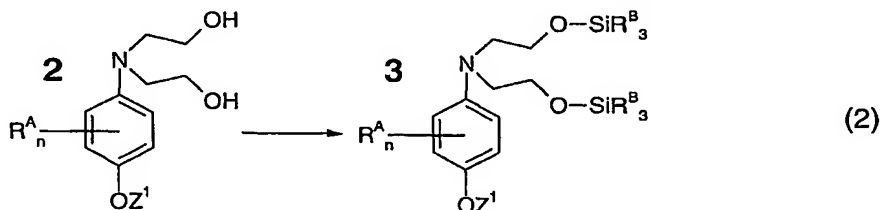
For example, the compound (or optionally an acid addition salt thereof) may be treated with ethylene oxide (oxirane), optionally in the presence of a suitable base, for example, triethylamine (NEt_3), and optionally in a suitable solvent.

- 5 Examples of suitable solvents include water, and protic and aprotic organic solvents. Examples of preferred solvents include acetic acid (MeCOOH , AcOH), alcohols, toluene, chloroform (CHCl_3), and benzene. In one preferred embodiment, the solvent is acetic acid.
- 10 In one embodiment, the molar ratio of the reagents $\text{ArNH}_2 \cdot \text{HCl}$: NEt_3 : ethylene oxide is about 1 : 1-1.5 : 5-20. In one embodiment, the solvent is used in an amount of about 5-20 mL/mmol of ArNH_2 . In one embodiment, the reaction time is about 3-120 h. In one embodiment, the reaction temperature is from about room temperature (e.g., about 20°C) to about 230°C . In one embodiment, the
- 15 reaction is carried out under stirring; a pressure vessel may be required if the reaction is carried out a temperature greater than about 40°C .

- In one preferred embodiment, the molar ratio of the reagents $\text{ArNH}_2 \cdot \text{HCl}$: NEt_3 : ethylene oxide is about 1 : 1.1 : 10; the solvent is acetic acid; the solvent is used
- 20 in an amount of about 10 mL/mmol of ArNH_2 ; the reaction time is about 72 h; and the reaction temperature is about room temperature; under stirring.

Silyloxy Protection (SP)

- 25 In this step, a 4-(protected hydroxy)-N,N-bis(2-hydroxyethyl)aniline, **2**, is converted to a 4-(protected hydroxy)-N,N-bis(2-silyloxyethyl)aniline, **3**.



- 26 -

The group $-\text{OSiR}^{\text{B}}_3$ is a silyloxy group (also known as a "silyl ether"), wherein each R^{B} is a silyl substituent, and is independently C_{1-7} alkyl, C_{3-20} heterocyclyl, or C_{5-20} aryl.

- 5 In one preferred embodiment, each R^{B} is independently C_{1-7} alkyl or C_{5-20} aryl. In one preferred embodiment, each R^{B} is independently saturated aliphatic C_{1-7} alkyl or C_{5-6} aryl. In one preferred embodiment, each R^{B} is independently -Me, -Et, -nPr, -iPr, -nBu, -sBu, -iBu, -tBu, or -Ph.
- 10 In one preferred embodiment, each group $-\text{SiR}^{\text{B}}_3$ is independently $-\text{Si}(\text{Me})_3$, $-\text{Si}(\text{Et})_3$, $-\text{Si}(\text{iPr})_3$, $-\text{Si}(\text{tBu})(\text{Me})_2$, or $-\text{Si}(\text{Ph})_2(\text{tBu})$. In one preferred embodiment, both $-\text{SiR}^{\text{B}}_3$ groups are the same. In one preferred embodiment, each $-\text{SiR}^{\text{B}}_3$ group is $-\text{Si}(\text{tBu})(\text{Me})_2$.
- 15 The conversion of the hydroxyl groups, -OH, to silyloxy groups, $-\text{OSiR}^{\text{B}}_3$, may be achieved using well known methods. See, for example, Lalonde et al., 1985 and Corey et al., 1972.

- For example, in one method, the compound is reacted with the corresponding
- 20 halosilane, $\text{SiR}^{\text{B}}_3\text{X}$, for example, the corresponding chlorosilane, $\text{SiR}^{\text{B}}_3\text{Cl}$, optionally in the presence of a suitable base, for example, imidazole, and optionally in a suitable solvent.

- Examples of suitable solvents include dry aprotic organic solvents. In one
- 25 preferred embodiment, the solvent is dimethylformamide (DMF).

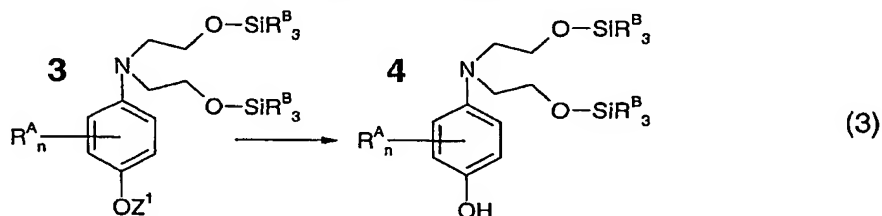
- In one embodiment, the molar ratio of the reagents diol (2) : $\text{ClSi}(\text{t-Bu})(\text{Me})_2$: imidazole is about 1 : 2-5 : 4-10. In one embodiment, the solvent is used in an amount of about 3-10 mL/mmol of diol. In one embodiment, the reaction time is
- 30 about 1-72 h. In one embodiment, the reaction temperature is about 0-100°C. In one embodiment, the reaction is carried out under stirring, under an inert gas (e.g., N_2 , argon).

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In one preferred embodiment, the molar ratio of the reagents diol (2) : ClSi(t-Bu)(Me)₂ : imidazole is about 1 : 2.5 : 4; the solvent is dimethylformamide; the solvent is used in an amount of about 3 mL/mmol of diol; the reaction time is about 18 h; the reaction temperature is about room temperature (e.g., about 20°C); under stirring, under an inert gas.

Phenolic Deprotection (PD)

10 In this step, a 4-(protected hydroxy)-N,N-bis(2-silyloxyethyl)aniline, **3**, is converted to a 4-[N,N-bis(2-silyloxyethyl)amino]phenol, **4**.



The conversion of the protected phenolic hydroxyl group, -OZ¹, to a phenolic hydroxyl group, -OH, may be achieved using well known methods, and will vary according to the particular protecting group, Z¹, as discussed above.

For example, in one method, when -OZ¹ is a benzyloxy group, optionally substituted on the aromatic ring, the compound may be treated with hydrogen gas (H₂) and a palladium/carbon catalyst (Pd/C), optionally in a suitable solvent.

20

Examples of suitable solvents include protic and aprotic organic solvents. Examples of preferred solvents include tetrahydrofuran (THF), ethanol (EtOH), and ethyl acetate (AcOEt). In one preferred embodiment, the solvent is tetrahydrofuran.

25

In one embodiment, the molar ratio of the reagents benzyl ether (3) : Pd/C : H₂ is about 1 : 0.05-0.5 (active Pd) : 5-20. In one embodiment, the catalyst is Pd/C

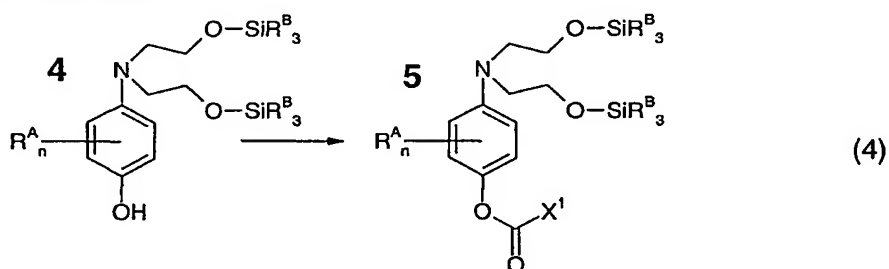
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5-30%. In one embodiment, the solvent is used in an amount of about 3-10 mL/mmol of benzyl ether. In one embodiment, the reaction time is about 1-48 h. In one embodiment, the reaction temperature is about 15-40°C. The reaction is carried out using hydrogenation apparatus; pressure may be applied if
 5 necessary.

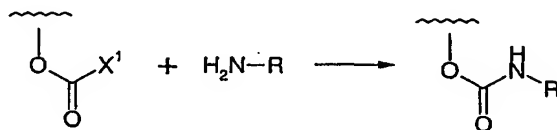
In one preferred embodiment, the molar ratio of the reagents benzyl ether (3) : Pd/C : H₂ is about 1 : 0.08 (active Pd) : 10; the catalyst is Pd/C 10%; the solvent is tetrahydrofuran; the solvent is used in an amount of about 5 mL/mmol of benzyl
 10 ether; the reaction time is about 24 h; the reaction temperature is about room temperature (e.g., about 20°C); using hydrogenation apparatus.

Activation (AC)

15 In this step, a 4-[N,N-bis(2-silyloxyethyl)amino]phenol, 4, is converted to a 4-[N,N-bis(2-silyloxyethyl)amino]phenyl haloformate, activated carbonate, or activated carbamate 5.



20 X¹ is a leaving group suitable for reaction with an amine to give a carbamate, as illustrated below,



and as discussed below in the glutamate conjugation (GC) step, i.e., where 5 + 15 gives a conjugate, 6.

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In one embodiment, the leaving group X^1 is (a) a halo group, and the group $-OC(=O)X^1$ is a haloformic ester group; (b) an activating group, $-OR$, and the group $-OC(=O)X^1$ is an activated carbonate; or (c) an activating group, $-NR_2$, and the group $-OC(=O)X^1$ is an activated carbamate.

5

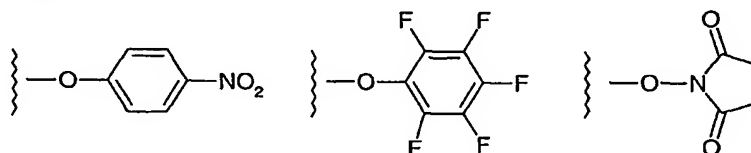
In one preferred embodiment, the leaving group X^1 is a halo group, and the group $-OC(=O)X^1$ is a haloformic ester group. In one preferred embodiment, the leaving group X^1 is an activating group, $-OR$, and the leaving group $-OC(=O)X^1$ is an activated carbonate. In one preferred embodiment, the leaving group X^1 is an activating group, $-NR_2$, and the group $-OC(=O)X^1$ is an activated carbamate.

10

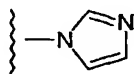
In one preferred embodiment, X^1 is $-F$, $-Cl$, $-Br$, *p*-nitrophenoxy, pentafluorophenoxy, succinimidyloxy, or imidazolyl.

15 In one preferred embodiment, X^1 is $-F$, $-Cl$, or $-Br$. In one preferred embodiment, X^1 is $-Cl$ or $-Br$. In one preferred embodiment, X^1 is $-F$ or $-Cl$. In one preferred embodiment, X^1 is $-F$. In one preferred embodiment, X^1 is $-Cl$. In one preferred embodiment, X^1 is $-Br$.

20 In one preferred embodiment, X^1 is *p*-nitrophenoxy, pentafluorophenoxy, or succinimidyloxy.



In one preferred embodiment, X^1 is imidazolyl.



25

- 30 -

The conversion of the phenolic hydroxyl group, -OH, to a haloformic ester, activated carbonate, or activated carbamate -O(C=O)X¹, may be achieved using well known methods. See, for example, Hagermann, 1983.

- 5 For example, in one method of converting to a haloformic ester, the compound is reacted with a carbonyl halide, X¹C(=O)X¹, for example, phosgene, ClC(=O)Cl, optionally in the presence of a suitable base (to combine with the HX¹, for example, HCl, formed during reaction), for example, triethylamine (NEt₃), and optionally in a suitable solvent.

10

Examples of suitable solvents include dry aprotic organic solvents. Examples of preferred solvents include tetrahydrofuran (THF) and toluene. In one preferred embodiment, the solvent is toluene.

- 15 In one embodiment, the molar ratio of the reagents phenol (4) : COCl₂ : NEt₃ is about 1 : 2-5 : 1-1.5. In one embodiment, the solvent is used in an amount of about 3-10 mL/mmol of phenol. In one embodiment, the reaction time is about 10 min to 24 h. In one embodiment, the reaction temperature is about 0-100°C. In one embodiment, the reaction is carried under stirring.

20

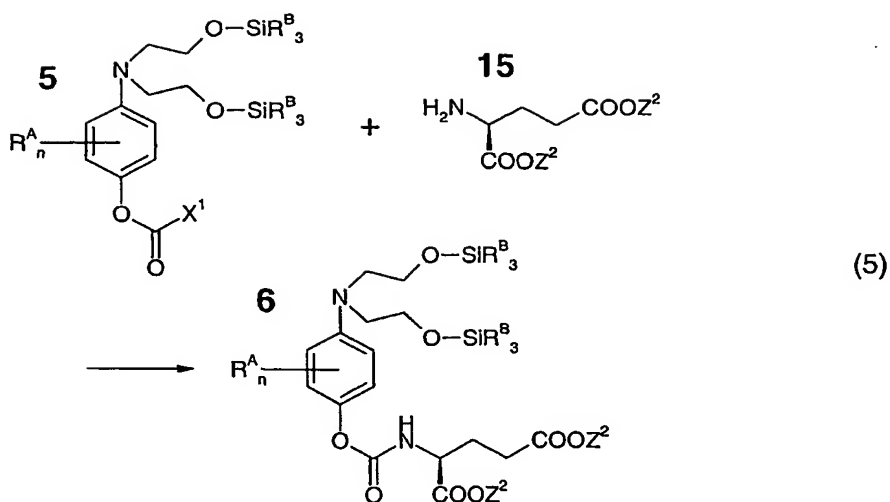
In one preferred embodiment, the molar ratio of the reagents phenol (4) : COCl₂ : NEt₃ is about 1 : 2.2 : 1.2; the solvent is toluene; the solvent is used in an amount of about 5 mL/mmol of phenol; the reaction time is about 10 min; the reaction temperature is about room temperature (e.g., about 20°C); under stirring.

25

Glutamate Conjugation (GC)

- In this step, a 4-[N,N-bis(2-silyloxyethyl)amino]phenyl haloformate, 5, reacts with a glutamate, 15, to give an N-{4-[N,N-bis(2-silyloxyethyl)amino]phenoxy-carbonyl}-L-glutamate, 6.
- 30

- 31 -



The formation of the carbamate linkage, $-\text{OC}(=\text{O})\text{NH}-$, from the haloformic ester, $-\text{O}(\text{C}=\text{O})\text{X}^1$, and the amino group, $-\text{NH}_2$ of the glutamate may be achieved using well known methods. See, for example, Periasamy et al., 1979 and Raucher et al., 1985.

For example, the two compounds may be mixed, optionally in the presence of a suitable base (to combine with the HX^1 , for example, HCl , formed during reaction), for example, triethylamine (NEt_3), and optionally in a suitable solvent.

The glutamate, **15**, may be provided in free form, or, more preferably, as an acid addition salt, e.g., **15.HCl**.

Examples of suitable solvents include aprotic organic solvents. In one preferred embodiment, the solvent is tetrahydrofuran (THF), toluene, or dichloromethane (DCM, CH_2Cl_2). In one preferred embodiment, the solvent is tetrahydrofuran.

In one embodiment, the molar ratio of the reagents chloroformate (**5**) : glutamate (**15**) : NEt_3 is about 1 : 0.8-1.2 : 1-2. In one embodiment, the solvent is used in an amount of about 3-10 mL/mmol of chloroformate. In one embodiment, the reaction time is about 10 min to 24 h. In one embodiment, the reaction

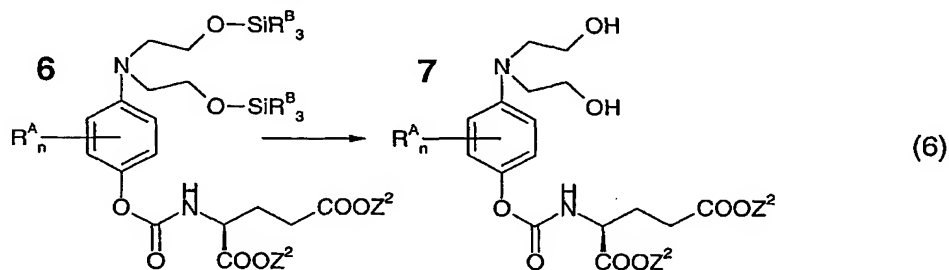
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temperature is about 0-70°C. In one embodiment, the reaction is carried out under stirring.

In one preferred embodiment, the molar ratio of the reagents chloroformate (5) : glutamate (15) : NEt_3 is about 1 : 1 : 1.1; the solvent is tetrahydrofuran; the solvent is used in an amount of about 5 mL/mmol of chloroformate; the reaction time is about 10 min; the reaction temperature is about room temperature (e.g., about 20°C); under stirring.

10 Siloxy Deprotection (SD)

In this step, an N-{4-[N,N-bis(2-silyloxyethyl) amino]phenoxy carbonyl}-L-glutamate, 6, is converted to an N-{4-[N,N-bis(2-hydroxyethyl)amino]phenoxy carbonyl}-L-glutamate, 7.



15

The conversion of the silyloxy group, $-\text{OSiR}_3$, to a hydroxyl groups, $-\text{OH}$, may be achieved using well known methods. See, for example, Greene et al., 1999b.

For example, in one method, the compound is reacted with triethylamine trihydrofluoride ($\text{NEt}_3 \cdot 3\text{HF}$), optionally in a suitable solvent.

20

Examples of suitable solvents include aprotic organic solvents. In one preferred embodiment, the solvent is tetrahydrofuran (THF), toluene, or dichloromethane (DCM, CH_2Cl_2). In one preferred embodiment, the solvent is tetrahydrofuran.

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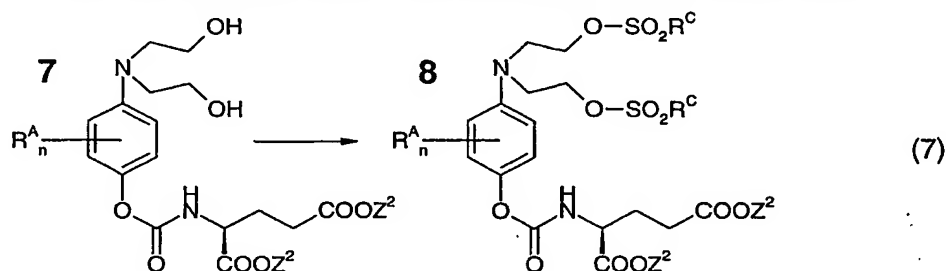
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In one embodiment, the molar ratio of the reagents bis-silyl ether (6) : $\text{NEt}_3 \cdot 3\text{HF}$ is about 1 : 5-20. In one embodiment, the solvent is used in an amount of about 5-20 mL/mmol of bis-silyl ether. In one embodiment, the reaction time is about 1-24 h. In one embodiment, the reaction temperature is about 0-40°C. In one
 5 embodiment, the reaction is carried out under stirring.

In one preferred embodiment, the molar ratio of the reagents bis-silyl ether (6) : $\text{NEt}_3 \cdot 3\text{HF}$ is about 1 : 10; the solvent is tetrahydrofuran; the solvent is used in an amount of about 11 mL/mmol of bis-silyl ether; the reaction time is about 12 h;
 10 the reaction temperature is about room temperature (e.g., about 20°C); under stirring.

Sulfonic Esterification (SU)

15 In this step, an N-{4-[N,N-bis(2-hydroxyethyl) amino]phenoxy carbonyl}-L-glutamate, 7, is converted to an N-{4-[N,N-bis(2-sulfonyloxyethyl)amino]phenoxy carbonyl}-L-glutamate, 8.



The R^{C} group of the sulfonic ester, $-\text{OSO}_2\text{R}^{\text{C}}$, is C_{1-7} alkyl, C_{3-20} heterocyclyl, or
 20 C_{5-20} aryl, and is optionally substituted. In one preferred embodiment, R^{C} is C_{1-7} alkyl or C_{5-20} aryl, and is optionally substituted.

In one preferred embodiment, R^{C} is C_{1-7} alkyl, and is optionally substituted. In one preferred embodiment, R^{C} is -Me, -Et, -nPr, -iPr, -nBu, -iBu, -sBu, or -tBu. In one
 25 preferred embodiment, R^{C} is -Me (and the group $-\text{SO}_2\text{R}^{\text{C}}$ is mesyl).

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In one preferred embodiment, R^C is phenyl or substituted phenyl. In one preferred embodiment, R^C is phenyl (and the group $-SO_2R^C$ is phenylsulfonyl). In one preferred embodiment, R^C is para-methylphenyl (and the group $-SO_2R^C$ is tosyl). In one preferred embodiment, R^C is para-bromophenyl (and the group $-SO_2R^C$ is brosyl). In one preferred embodiment, R^C is para-nitrophenyl (and the group $-SO_2R^C$ is nosyl).

The conversion of the hydroxy groups, $-OH$, to sulfonic ester groups, $-OSO_2R^C$, may be achieved using well known methods. See, for example, Hagermann, 1985.

For example, in one method, the compound is reacted with sulfonic anhydride, $(R^CSO_2)_2O$, for example, methanesulfonic anhydride, $(MeSO_2)_2O$, optionally in the presence of one or more bases, for example, triethylamine (NEt_3) and/or 4-dimethylaminopyridine (DMAP), optionally in a suitable solvent.

Examples of suitable solvents include dry aprotic organic solvents. In one preferred embodiment, the solvent is dichloromethane (DCM , CH_2Cl_2), tetrahydrofuran (THF), ethyl acetate ($AcOEt$), or toluene. In one preferred embodiment, the solvent is dichloromethane.

In one embodiment, the molar ratio of the reagents diol (7) : $(MeSO_2)_2O$: NEt_3 : DMAP is about 1 : 2-10 : 2-10 : 0.05-2. In one embodiment, the solvent is used in an amount of about 3-20 mL/mmol of diol. In one embodiment, the reaction time is about 1-48 h. In one embodiment, the reaction temperature is about 0-50°C. In one embodiment, the reaction is carried out in an ice bath. In one embodiment, the reaction is carried out under stirring.

In one preferred embodiment, the molar ratio of the reagents diol (7) : $(MeSO_2)_2O$: NEt_3 : DMAP is about 1 : 4.3 : 5.4 : 0.07; the solvent is dichloromethane; the solvent is used in an amount of about 3 mL/mmol of diol; the reaction time is

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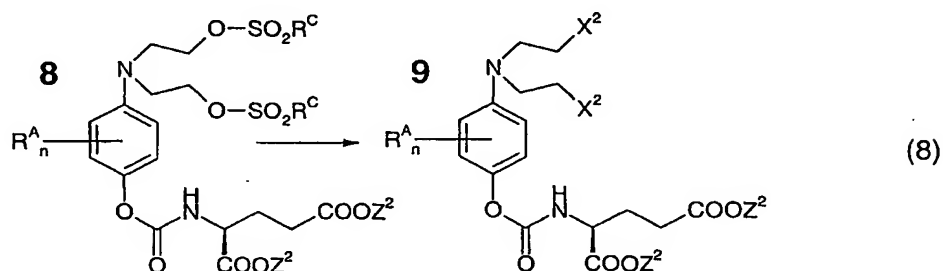
about 18 h; the reaction starts at 0°C and is allowed to warm at room temperature; under stirring.

Halogenation (HL)

5

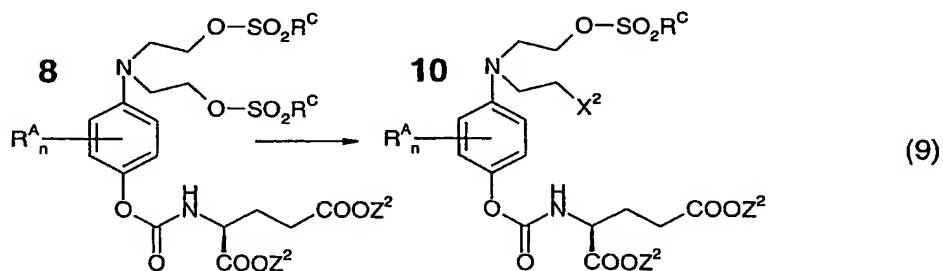
In this step, an N-{4-[N,N-bis(2-sulfonyloxyethyl) amino]phenoxy carbonyl}-L-glutamate, **8**, is converted to either:

(a) a dihalo product, N-{4-[N,N-bis(2-haloethyl) amino] phenoxy carbonyl}-

10 L-glutamate, **9**.

or:

(b) a mixed halo-sulfonyl product, N-{4-[N-(2-sulfonyloxy

15 ethyl),N-(2-haloethyl)amino]phenoxy carbonyl}-L-glutamate, **10**.

In one preferred embodiment, the reaction gives the dihalo product, **9**.

Each group X^2 is independently -F, -Cl, -Br, -I. In one preferred embodiment,

20 each X^2 is independently -Cl, -Br, or -I. In one preferred embodiment, each X^2 is

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independently -Cl or -I. In one preferred embodiment, each X^2 is independently -Cl or -Br. In one preferred embodiment, each X^2 is independently -Br or -I. In one preferred embodiment, each X^2 is -F. In one preferred embodiment, each X^2 is -Cl. In one preferred embodiment, each X^2 is -Br. In one preferred
5 embodiment, each X^2 is -I. In one embodiment, when the product is a dihalo product, both X^2 groups are the same (e.g., difluoro, dichloro, dibromo, diiodo).

For the mixed halo-sulfonyl products, **10**, a preferred sulfonyloxy group, -OSO₂R^C, is -OSO₂Me.

10

The conversion of the sulfonyloxy groups, -OSO₂R^C, to halo groups, -X², may be achieved using well known methods. See, for example, March, 1992.

For example, in one method, the compound is reacted with alkaline halide, for
15 example, sodium halide, for example, sodium iodide, optionally in a suitable solvent.

Examples of suitable solvents include aprotic organic solvents. In one preferred embodiment, the solvent is tetrahydrofuran (THF), acetone, dimethylformamide (DMF), acetonitrile (ACN), or dimethylacetamide. In one preferred embodiment,
20 the solvent is acetone.

Examples of suitable couples of alkaline halide/solvent include, for example, NaI/acetone, LiBr/tetrahydrofuran, and LiCl/dimethylacetamide. In one preferred
25 embodiment, the alkaline halide/solvent couple is NaI/acetone.

In one embodiment, the molar ratio of the reagents mesylate (**8**) : NaI is about 1 : 10-20. In one embodiment, the solvent is used in an amount of about 10-50 mL/mmol of mesylate. In one embodiment, the reaction time is about 1-24 h.
30 In one embodiment, the reaction temperature is about 20-100°C. In one embodiment, the reaction is carried out using a condenser. In one embodiment, the reaction is carried out under stirring.

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In one preferred embodiment, the molar ratio of the reagents mesylate (8) : NaI is about 1 : 13; the solvent is acetone; the solvent is used in an amount of about 30 mL/mmol of mesylate; the reaction time is about 4.5 h; the reaction temperature is at reflux; under stirring.

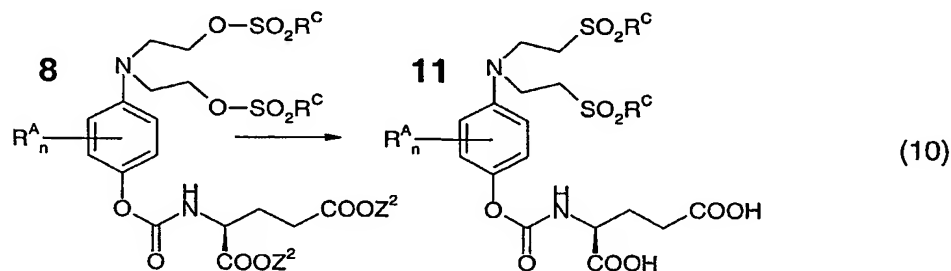
If the mixed halo-sulfonyl product is desired, the ratio of alkaline halide : bis-sulfonyl compounds is reduced to 0.5-1 : 1, the reaction is stopped before completion, and the halo-sulfonyl product is isolated from the reaction mixture.

An alternative method uses methanesulfonyl chloride and pyridine at an intermediate temperature; the reaction is stopped before completion and the halo-sulfonyl product is isolated from the reaction mixture. See, for example, Springer et al., 1994, Schemes 1 and 2 on page 12-13 therein, and Springer et al., 1995, compound 1c therein.

Glutamate Deprotection (GD)

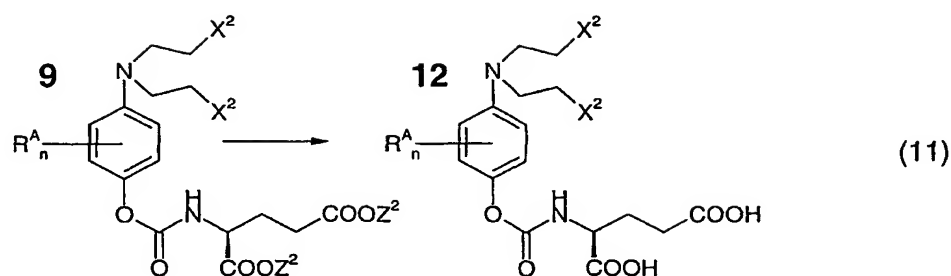
In this step, one of the following three reactions is performed:

(a) a N-{4-[N,N-bis(2-sulfonyloxyethyl)amino] phenoxy carbonyl} -L-glutamate, **8**, is converted to a N-{4-[N,N-bis(2-sulfonyl oxyethyl)amino]phenoxy carbonyl}-L-glutamic acid, **11**:

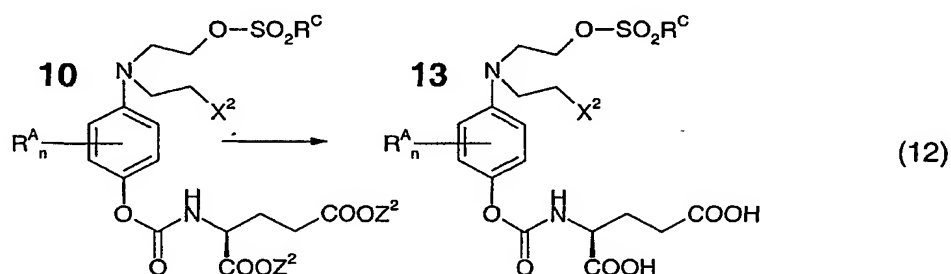


(b) an N-{4-[N,N-bis(2-haloethyl)amino] phenoxy carbonyl}-L-glutamate, **9**, is converted to an N-{4-[N,N-bis (2-haloethyl)amino]phenoxy carbonyl}-L-glutamic acid, **12**,

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(c) a N-{4-[N-(2-sulfonyloxy ethyl),N-(2-haloethyl)amino] phenoxy carbonyl}-L-glutamate, **10**, is converted to a deprotected mixed halo-sulfonyl product, N-{4-[N-(2-sulfonyloxyethyl),N-(2-haloethyl)amino] phenoxy carbonyl}-L-glutamic acid, **13**.



In one preferred embodiment, the reaction involves the dihalo ester, **9**, and gives the dihalo acid, **12**.

10 The glutamate ester groups, $-\text{COOZ}^2$, may be converted to acid groups, $-\text{COOH}$, using well known methods, and will vary according to the particular protecting group, Z^2 . See, for example, Greene et al., 1999c.

For example, in one method, when $-\text{Z}^2$ is t-butyl, the compound may be treated
15 with a suitable acid, for example, trifluoroacetic acid (CF_3COOH , TFA), optionally in a suitable solvent.

In one preferred embodiment, the solvent is trifluoroacetic acid.

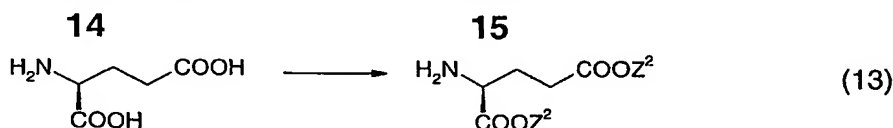
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In one embodiment, the molar ratio of the reagents ester (9) : TFA is about 1 : 100-1000. In one embodiment, the solvent is used in an amount of about 5-50 mL/mmol of ester. In one embodiment, the reaction time is about 30 min to 3 h. In one embodiment, the reaction temperature is about 0-40°C. In one
 5 embodiment, the reaction is carried out under stirring.

In one preferred embodiment, the molar ratio of the reagents ester (8, 9 or 10): TFA is about 1 : 500; the solvent is (also) TFA; the solvent is used in an amount of about 18 mL/mmol of ester; the reaction time is about 55 min; the reaction
 10 temperature is about room temperature (e.g., about 20°C); under stirring.

Glutamate Protection (GP)

In this (parallel) step, L-glutamic acid, **14**, is converted to an L-glutamate, **15**.



15

The acid groups, -COOH, of L-glutamate may be protected as esters, -COOZ², using well known methods (Greene et al, 1999c). For t-butyl esters, see for example, Ferenz et al., 1989. The group -COOZ² is a protected carboxylic acid group. The group, -Z² is a suitable carboxylic acid protecting group. In one
 20 preferred embodiment, each of the groups Z² may independently be t-butyl, allyl, or benzyl. In one preferred embodiment, each of the groups Z² is t-butyl.

For example, in one method, L-glutamic acid is reacted with isobutylene, in the presence of an acidic catalyst, for example, sulfuric acid, and optionally in a
 25 suitable solvent.

Examples of suitable solvents include aprotic organic solvents. In one preferred embodiment, the solvent is chloroform (CHCl₃) or dichloromethane (DCM, CH₂Cl₂). In one preferred embodiment, the solvent is chloroform.

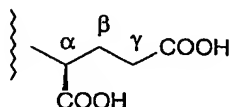
- 40 -

In one embodiment, the molar ratio of the reagents glutamic acid (14) : H_2SO_4 : isobutylene is about 1 : 1.2-2 : 20-150. In one embodiment, the solvent is used in an amount of about 3-20 mL/mmol of glutamic acid. In one embodiment, the
 5 reaction time is about 18-120 h. In one embodiment, the reaction temperature is about -70°C to about 25°C . In one embodiment, the reaction is carried out using an acetone-solid CO_2 bath, and optionally in a pressure vessel.

In one preferred embodiment, the molar ratio of the reagents glutamic acid (14) : H_2SO_4 : isobutylene is about 1 : 1.8 : 25; the solvent is chloroform; the solvent is
 10 used in an amount of about 4 mL/mmol of glutamic acid; the reaction time is about 18 h; the reaction temperature starts at -78°C and is allowed to warm at room temperature.

15 Alternatives to the L-Glutamic Acid Moiety

The invention is described herein in detail in regard to embodiments involving an L-glumatic acid moiety:

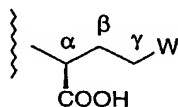


20

Nonetheless, in one embodiment, this group is generalised to include corresponding analogues. Examples of such analogues are described in Burke et al., 1994. Particularly preferred analogues includes those described at page 3-5 and 7 in Burke et al., 1994 (WO 94/02450).

25

In one embodiment, this group is generalised to include corresponding γ -analogues:



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wherein W is, for example, a group denoted W as described on page 7 of Burke et al., 1994 (WO 94/02450).

In one embodiment, W is -COOH, -CONH₂, -CONHPh, or tetrazolyl.

5

In one embodiment, the glutamic acid moiety has a D-configuration, instead of an L-configuration.

In one embodiment, the L-glutamic acid moiety is generalised to include other amino acid groups, for example, aspartic acid, and amino acid oligomers, for example, Glu-Glu.

10

Chemical Terms

15 The term "carbo," "carbyl," "hydrocarbo," and "hydrocarbyl," as used herein, pertain to compounds and/or groups which have only carbon and hydrogen atoms.

The term "hetero," as used herein, pertains to compounds and/or groups which have at least one heteroatom, for example, multivalent heteroatoms (which are also suitable as ring heteroatoms) such as boron, silicon, nitrogen, phosphorus, oxygen, and sulfur, and monovalent heteroatoms, such as fluorine, chlorine, bromine, and iodine.

20

25 The term "saturated," as used herein, pertains to compounds and/or groups which do not have any carbon-carbon double bonds or carbon-carbon triple bonds.

The term "unsaturated," as used herein, pertains to compounds and/or groups which have at least one carbon-carbon double bond or carbon-carbon triple bond.

30

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The term "aliphatic," as used herein, pertains to compounds and/or groups which are linear or branched, but not cyclic (also known as "acyclic" or "open-chain" groups).

- 5 The term "cyclic," as used herein, pertains to compounds and/or groups which have one ring, or two or more rings (e.g., spiro, fused, bridged). Compounds with one ring may be referred to as "monocyclic" or "mononuclear," whereas compounds with two or more rings may be referred to as "polycyclic" or "polynuclear."

10

The term "ring," as used herein, pertains to a closed ring of from 3 to 10 covalently linked atoms, more preferably 3 to 8 covalently linked atoms.

- 15 The term "aromatic ring," as used herein, pertains to a closed ring of from 3 to 10 covalently linked atoms, more preferably 5 to 8 covalently linked atoms, more preferably 5 to 6 covalently linked atoms, which ring is aromatic.

- 20 The term "heterocyclic ring," as used herein, pertains to a closed ring of from 3 to 10 covalently linked atoms, more preferably 3 to 8 covalently linked atoms, wherein at least one of the ring atoms is a multivalent ring heteroatom, for example, nitrogen, phosphorus, silicon, oxygen, and sulfur, though more commonly nitrogen, oxygen, and sulfur.

- 25 The term "alicyclic," as used herein, pertains to compounds and/or groups which have one ring, or two or more rings (e.g., spiro, fused, bridged), wherein said ring(s) are not aromatic.

The term "aromatic," as used herein, pertains to compounds and/or groups which have one ring, or two or more rings (e.g., fused), wherein said ring(s) are aromatic.

30

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The term "heterocyclic," as used herein, pertains to cyclic compounds and/or groups which have one heterocyclic ring, or two or more heterocyclic rings (e.g., spiro, fused, bridged), wherein said ring(s) may be alicyclic or aromatic.

- 5 The term "heteroaromatic," as used herein, pertains to cyclic compounds and/or groups which have one heterocyclic ring, or two or more heterocyclic rings (e.g., fused), wherein said ring(s) is aromatic.

- 10 The phrase "optionally substituted," as used herein, pertains to a parent group which may be unsubstituted or which may be substituted.

- 15 Unless otherwise specified, the term "substituted," as used herein, pertains to a parent group which bears one or more substituents. The term "substituent" is used herein in the conventional sense and refers to a chemical moiety which is covalently attached to, appended to, or if appropriate, fused to, a parent group. A wide variety of substituents are well known, and methods for their formation and introduction into a variety of parent groups are also well known.

- 20 Various substituents are described in more detail below.

- 25 **C₁₋₇alkyl:** The term "C₁₋₇alkyl," as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from a C₁₋₇hydrocarbon compound having from 1 to 7 carbon atoms, which may be aliphatic or alicyclic, or a combination thereof, and which may be saturated, partially unsaturated, or fully unsaturated.

Examples of (unsubstituted) saturated linear C₁₋₇alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, n-butyl, and n-pentyl (amyl).

- 30 Examples of (unsubstituted) saturated branched C₁₋₇alkyl groups include, but are not limited to, iso-propyl, iso-butyl, sec-butyl, tert-butyl, and neo-pentyl.

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Examples of saturated alicyclic (carbocyclic) C₁₋₇alkyl groups (also referred to as "C₃₋₇cycloalkyl" groups) include, but are not limited to, unsubstituted groups such as cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl, as well as substituted groups (e.g., groups which comprise such groups), such as methylcyclopropyl, dimethylcyclopropyl, methylcyclobutyl, dimethylcyclobutyl, methylcyclopentyl, dimethylcyclopentyl, methylcyclohexyl, dimethylcyclohexyl, cyclopropylmethyl and cyclohexylmethyl.

Examples of (unsubstituted) unsaturated C₁₋₇alkyl groups which have one or more carbon-carbon double bonds (also referred to as "C₂₋₇alkenyl" groups) include, but are not limited to, ethenyl (vinyl, -CH=CH₂), 2-propenyl (allyl, -CH-CH=CH₂), isopropenyl (-C(CH₃)=CH₂), butenyl, pentenyl, and hexenyl.

Examples of (unsubstituted) unsaturated C₁₋₇alkyl groups which have one or more carbon-carbon triple bonds (also referred to as "C₂₋₇alkynyl" groups) include, but are not limited to, ethynyl (ethinyl) and 2-propynyl (propargyl).

Examples of unsaturated alicyclic (carbocyclic) C₁₋₇alkyl groups which have one or more carbon-carbon double bonds (also referred to as "C₃₋₇cycloalkenyl" groups) include, but are not limited to, unsubstituted groups such as cyclopropenyl, cyclobutenyl, cyclopentenyl, and cyclohexenyl, as well as substituted groups (e.g., groups which comprise such groups) such as cyclopropenylmethyl and cyclohexenylmethyl.

C₃₋₂₀heterocyclyl: The term "C₃₋₂₀heterocyclyl," as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from a ring atom of a C₃₋₂₀heterocyclic compound, said compound having one ring, or two or more rings (e.g., spiro, fused, bridged), and having from 3 to 20 ring atoms, atoms, of which from 1 to 10 are ring heteroatoms, and wherein at least one of said ring(s) is a heterocyclic ring. Preferably, each ring has from 3 to 7 ring atoms, of which from 1 to 4 are ring heteroatoms. "C₃₋₂₀" denotes ring atoms, whether carbon atoms or heteroatoms.

Examples of (non-aromatic) C₃₋₂₀heterocyclyl groups having one nitrogen ring atom include, but are not limited to, those derived from aziridine, azetidine, azetine, pyrrolidine, pyrroline, piperidine, dihydropyridine, tetrahydropyridine, and dihydropyrrole (azoline).

Examples of (non-aromatic) C₃₋₂₀heterocyclyl groups having one oxygen ring atom include, but are not limited to, those derived from oxirane, oxetane, oxolane (tetrahydrofuran), oxole (dihydrofuran), oxane (tetrahydropyran), dihydropyran, and pyran. Examples of substituted C₃₋₂₀heterocyclyl groups include sugars, in cyclic form, for example, furanoses and pyranoses, including, for example, ribose, lyxose, xylose, galactose, sucrose, fructose, and arabinose.

Examples of (non-aromatic) C₃₋₂₀heterocyclyl groups having one sulfur ring atom include, but are not limited to, those derived from thiolane (tetrahydrothiophene, thiane) and tetrahydrothiopyran.

Examples of (non-aromatic) C₃₋₂₀heterocyclyl groups having two oxygen ring atoms include, but are not limited to, those derived from dioxane.

Examples of (non-aromatic) C₃₋₂₀heterocyclyl groups having two nitrogen ring atoms include, but are not limited to, those derived from diazolidine (pyrazolidine), pyrazoline, imidazolidine, imidazoline, and piperazine.

Examples of (non-aromatic) C₃₋₂₀heterocyclyl groups having one nitrogen ring atom and one oxygen ring atom include, but are not limited to, those derived from tetrahydrooxazole, dihydrooxazole, tetrahydroisoxazole, dihydroisoxazole, morpholine, tetrahydrooxazine, dihydrooxazine, and oxazine.

Examples of (non-aromatic) C₃₋₂₀heterocyclyl groups having one oxygen ring atom and one sulfur ring atom include, but are not limited to, those derived from oxathiolane and oxathiane.

Examples of (non-aromatic) C₃₋₂₀heterocyclyl groups having one nitrogen ring atom and one sulfur ring atom include, but are not limited to, those derived from thiazoline, thiazolidine, and thiomorpholine.

5

Other examples of (non-aromatic) C₃₋₂₀heterocyclyl groups include, but are not limited to, oxadiazine.

10 C₅₋₂₀aryl: The term "C₅₋₂₀aryl," as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from an aromatic ring atom of a C₅₋₂₀aromatic compound, said compound having one ring, or two or more rings (e.g., fused), and having from 5 to 20 ring atoms, and wherein at least one of said ring(s) is an aromatic ring. Preferably, each ring has from 5 to 7 ring atoms.

15 The ring atoms may be all carbon atoms, as in "carboaryl groups," in which case the group may conveniently be referred to as a "C₅₋₂₀carboaryl" group.

Examples of C₅₋₂₀aryl groups which do not have ring heteroatoms (i.e., C₅₋₂₀carboaryl groups) include, but are not limited to, those derived from
20 benzene (i.e., phenyl) (C₆), naphthalene (C₁₀), anthracene (C₁₄), phenanthrene (C₁₄), pyrene (C₁₆), and naphthacene (C₁₈).

Alternatively, the ring atoms may include one or more heteroatoms, including but not limited to oxygen, nitrogen, and sulfur, as in "heteroaryl groups." In this case,
25 the group may conveniently be referred to as a "C₅₋₂₀heteroaryl" group, wherein "C₅₋₂₀" denotes ring atoms, whether carbon atoms or heteroatoms. Preferably, each ring has from 5 to 7 ring atoms, of which from 0 to 4 are ring heteroatoms.

Examples of C₅₋₂₀heteroaryl groups include, but are not limited to, C₅heteroaryl
30 groups derived from furan (oxole), thiophene (thiole), pyrrole (azole), imidazole (1,3-diazole), pyrazole (1,2-diazole), triazole, oxazole, isoxazole, thiazole, isothiazole, oxadiazole, and oxatriazole; and C₆heteroaryl groups derived from

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isoxazine, pyridine (azine), pyridazine (1,2-diazine), pyrimidine (1,3-diazine; e.g., cytosine, thymine, uracil), pyrazine (1,4-diazine), triazine, tetrazole, and oxadiazole (furazan).

- 5 Examples of C₅₋₂₀heterocyclic groups (including C₅₋₂₀heteroaryl groups) which comprise fused rings, include, but are not limited to, C₉heterocyclic groups derived from benzofuran, isobenzofuran, indole, isoindole, purine (e.g., adenine, guanine), benzimidazole; C₁₀heterocyclic groups derived from quinoline, isoquinoline, benzodiazine, pyridopyridine, quinoxaline; C₁₃heterocyclic groups
10 derived from carbazole; C₁₄heterocyclic groups derived from acridine, xanthene, phenoxathiin, phenazine, phenoxazine, phenothiazine.

The above C₁₋₇alkyl, C₃₋₂₀heterocyclyl, and C₅₋₂₀aryl groups, whether alone or part of another substituent, may themselves optionally be substituted with one or
15 more groups selected from themselves and the additional substituents listed below.

Hydrogen: -H. Note that if the substituent at a particular position is hydrogen, it may be convenient to refer to the compound as being "unsubstituted" at that
20 position.

Halo: -F, -Cl, -Br, and -I.

Hydroxy: -OH.

25

Ether: -OR, wherein R is an ether substituent, for example, a C₁₋₇alkyl group (also referred to as a C₁₋₇alkoxy group, discussed below), a C₃₋₂₀heterocyclyl group (also referred to as a C₃₋₂₀heterocyclyloxy group), or a C₅₋₂₀aryl group (also referred to as a C₅₋₂₀aryloxy group), preferably a C₁₋₇alkyl group.

30

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C₁₋₇alkoxy: -OR, wherein R is a C₁₋₇alkyl group. Examples of C₁₋₇alkoxy groups include, but are not limited to, -OCH₃ (methoxy), -OCH₂CH₃ (ethoxy) and -OC(CH₃)₃ (tert-butoxy).

5 Formyl (carbaldehyde, carboxaldehyde): -C(=O)H.

Acyl (keto): -C(=O)R, wherein R is an acyl substituent, for example, a C₁₋₇alkyl group (also referred to as C₁₋₇alkylacyl or C₁₋₇alkanoyl), a C₃₋₂₀heterocyclyl group (also referred to as C₃₋₂₀heterocyclylacyl), or a C₅₋₂₀aryl group (also referred to as C₅₋₂₀arylacyl), preferably a C₁₋₇alkyl group. Examples of acyl groups include, but are not limited to, -C(=O)CH₃ (acetyl), -C(=O)CH₂CH₃ (propionyl), -C(=O)C(CH₃)₃ (butyryl), and -C(=O)Ph (benzoyl, phenone).

10

Acylhalide (haloformyl, halocarbonyl): -C(=O)X, wherein X is -F, -Cl, -Br, or -I, preferably -Cl, -Br, or -I.

15

Carboxy (carboxylic acid): -COOH.

Ester (carboxylate, carboxylic acid ester, oxycarbonyl): -C(=O)OR, wherein R is an ester substituent, for example, a C₁₋₇alkyl group, a C₃₋₂₀heterocyclyl group, or a C₅₋₂₀aryl group, preferably a C₁₋₇alkyl group. Examples of ester groups include, but are not limited to, -C(=O)OCH₃, -C(=O)OCH₂CH₃, -C(=O)OC(CH₃)₃, and -C(=O)OPh.

20

Acyloxy (reverse ester): -OC(=O)R, wherein R is an acyloxy substituent, for example, a C₁₋₇alkyl group, a C₃₋₂₀heterocyclyl group, or a C₅₋₂₀aryl group, preferably a C₁₋₇alkyl group. Examples of acyloxy groups include, but are not limited to, -OC(=O)CH₃ (acetoxyl), -OC(=O)CH₂CH₃, -OC(=O)C(CH₃)₃, -OC(=O)Ph, and -OC(=O)CH₂Ph.

25

30

Amido (carbamoyl, carbamyl, aminocarbonyl, carboxamide): -C(=O)NR¹R², wherein R¹ and R² are independently amino substituents, as defined for amino

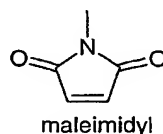
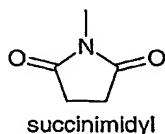
groups. Examples of amido groups include, but are not limited to, $-C(=O)NH_2$, $-C(=O)NHCH_3$, $-C(=O)NH(CH_3)_2$, $-C(=O)NHCH_2CH_3$, and $-C(=O)N(CH_2CH_3)_2$, as well as amido groups in which R^1 and R^2 , together with the nitrogen atom to which they are attached, form a heterocyclic structure as in, for example,

5 piperidinocarbonyl, morpholinocarbonyl, thiomorpholinocarbonyl, and piperazinocarbonyl.

Acylamido (acylamino): $-NR^1C(=O)R^2$, wherein R^1 is an amide substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group, and R^2 is an acyl substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group.

Examples of acylamide groups include, but are not limited to, $-NHC(=O)CH_3$, $-NHC(=O)CH_2CH_3$, and $-NHC(=O)Ph$. R^1 and R^2 may together form a cyclic structure, as in, for example, succinimidyl and maleimidyl:

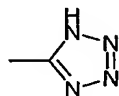
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Thioamido (thiocarbamyl): $-C(=S)NR^1R^2$, wherein R^1 and R^2 are independently amino substituents, as defined for amino groups. Examples of amido groups include, but are not limited to, $-C(=S)NH_2$, $-C(=S)NHCH_3$, $-C(=S)NH(CH_3)_2$, and

20 $-C(=S)NHCH_2CH_3$.

Tetrazolyl: a five membered aromatic ring having four nitrogen atoms and one carbon atom,



25

Amino: $-NR^1R^2$, wherein R^1 and R^2 are independently amino substituents, for example, hydrogen, a C_{1-7} alkyl group (also referred to as C_{1-7} alkylamino or di- C_{1-7} alkylamino), a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably H or

- 50 -

a C₁₋₇alkyl group, or, in the case of a "cyclic" amino group, R¹ and R², taken together with the nitrogen atom to which they are attached, form a heterocyclic ring having from 4 to 8 ring atoms. Examples of amino groups include, but are not limited to, -NH₂, -NHCH₃, -NHCH(CH₃)₂, -N(CH₃)₂, -N(CH₂CH₃)₂, and -NHPh.

- 5 Examples of cyclic amino groups include, but are not limited to, aziridino, azetidino, piperidino, piperazino, morpholino, and thiomorpholino.

Nitro: -NO₂.

- 10 Nitroso: -NO.

Azido: -N₃.

Cyano (nitrile, carbonitrile): -CN.

15

Isocyano: -NC.

Cyanato: -OCN.

- 20 Isocyanato: -NCO.

Thiocyano (thiocyanato): -SCN.

Isothiocyano (isothiocyano): -NCS.

25

Sulfhydryl (thiol, mercapto): -SH.

- Thioether (sulfide): -SR, wherein R is a thioether substituent, for example, a C₁₋₇alkyl group (also referred to as a C₁₋₇alkylthio group), a C₃₋₂₀heterocyclyl group (also referred to as a C₃₋₂₀heterocyclylthio group), or a C₅₋₂₀aryl group (also referred to as a C₅₋₂₀arylthio group), preferably a C₁₋₇alkyl group. Examples of C₁₋₇alkylthio groups include, but are not limited to, -SCH₃ and -SCH₂CH₃.
- 30

Sulfonic acid (sulfo): $-S(=O)_2OH$.

- 5 Sulfonate (sulfonic acid ester): $-S(=O)_2OR$, wherein R is a sulfonate substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfonate groups include, but are not limited to, $-S(=O)_2OCH_3$ and $-S(=O)_2OCH_2CH_3$.

- 10 Sulfone (sulfonyl): $-S(=O)_2R$, wherein R is a sulfone substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfone groups include, but are not limited to, $-S(=O)_2CH_3$ (methanesulfonyl, mesyl), $-S(=O)_2CF_3$, $-S(=O)_2CH_2CH_3$, and 4-methylphenylsulfonyl (tosyl).

- 15 Sulfonyloxy: $-OS(=O)_2R$, wherein R is a sulfonyloxy substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfonyloxy groups include, but are not limited to, $-OS(=O)_2CH_3$ and $-OS(=O)_2CH_2CH_3$.

- 20 Sulfinyloxy: $-OS(=O)R$, wherein R is a sulfinyloxy substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfinyloxy groups include, but are not limited to, $-OS(=O)CH_3$ and $-OS(=O)CH_2CH_3$.

- 25 Sulfamino: $-NR^1S(=O)_2OH$, wherein R^1 is an amino substituent, as defined for amino groups. Examples of sulfamino groups include, but are not limited to, $-NHS(=O)_2OH$ and $-N(CH_3)S(=O)_2OH$.

- 30 Sulfonamino: $-NR^1S(=O)_2R$, wherein R^1 is an amino substituent, as defined for amino groups, and R is a sulfonamino substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group.

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Examples of sulfonamino groups include, but are not limited to, $\text{-NHS(=O)}_2\text{CH}_3$ and $\text{-N(CH}_3\text{)S(=O)}_2\text{C}_6\text{H}_5$.

5 Sulfenamino: $\text{-NR}^1\text{S(=O)R}$, wherein R^1 is an amino substituent, as defined for amino groups, and R is a sulfenamino substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfenamino groups include, but are not limited to, -NHS(=O)CH_3 and $\text{-N(CH}_3\text{)S(=O)C}_6\text{H}_5$.

10 Sulfamyl: $\text{-S(=O)NR}^1\text{R}^2$, wherein R^1 and R^2 are independently amino substituents, as defined for amino groups. Examples of sulfamyl groups include, but are not limited to, -S(=O)NH_2 , $\text{-S(=O)NH(CH}_3\text{)}$, $\text{-S(=O)N(CH}_3\text{)}_2$, $\text{-S(=O)NH(CH}_2\text{CH}_3\text{)}$, $\text{-S(=O)N(CH}_2\text{CH}_3\text{)}_2$, and -S(=O)NHPh .

15 Sulfonamido: $\text{-S(=O)}_2\text{NR}^1\text{R}^2$, wherein R^1 and R^2 are independently amino substituents, as defined for amino groups. Examples of sulfonamido groups include, but are not limited to, $\text{-S(=O)}_2\text{NH}_2$, $\text{-S(=O)}_2\text{NH(CH}_3\text{)}$, $\text{-S(=O)}_2\text{N(CH}_3\text{)}_2$, $\text{-S(=O)}_2\text{NH(CH}_2\text{CH}_3\text{)}$, $\text{-S(=O)}_2\text{N(CH}_2\text{CH}_3\text{)}_2$, and $\text{-S(=O)}_2\text{NHPh}$.

20 For example, a C_{1-7} alkyl group may be substituted with, for example, hydroxy (also referred to as a C_{1-7} hydroxyalkyl group), C_{1-7} alkoxy (also referred to as a C_{1-7} alkoxyalkyl group), amino (also referred to as a C_{1-7} aminoalkyl group), halo (also referred to as a C_{1-7} haloalkyl group), carboxy (also referred to as a C_{1-7} carboxyalkyl group), and C_{5-20} aryl (also referred to as a C_{5-20} aryl- C_{1-7} alkyl group).
25

Similarly, a C_{5-20} aryl group may be substituted with, for example, hydroxy (also referred to as a C_{5-20} hydroxyaryl group), halo (also referred to as a C_{5-20} haloaryl group), amino (also referred to as a C_{5-20} aminoaryl group, e.g., as in aniline),
30 C_{1-7} alkyl (also referred to as a C_{1-7} alkyl- C_{5-20} aryl group, e.g., as in toluene), and C_{1-7} alkoxy (also referred to as a C_{1-7} alkoxy- C_{5-20} aryl group, e.g., as in anisole).

These and other specific examples of such substituted groups are also discussed below.

C₁₋₇haloalkyl group: The term "C₁₋₇haloalkyl group," as used herein, pertains to a
5 C₁₋₇alkyl group in which at least one hydrogen atom (e.g., 1, 2, 3) has been replaced with a halogen atom (e.g., F, Cl, Br, I). If more than one hydrogen atom has been replaced with a halogen atom, the halogen atoms may independently be the same or different. Every hydrogen atom may be replaced with a halogen atom, in which case the group may conveniently be referred to as a
10 C₁₋₇perhaloalkyl group." Examples of C₁₋₇haloalkyl groups include, but are not limited to, -CF₃, -CHF₂, -CH₂F, -CCl₃, -CBr₃, -CH₂CH₂F, -CH₂CHF₂, and -CH₂CF₃.

C₁₋₇haloalkoxy: -OR, wherein R is a C₁₋₇haloalkyl group. Examples of
C₁₋₇haloalkoxy groups include, but are not limited to, -OCF₃, -OCHF₂, -OCH₂F,
15 -OCCl₃, -OCBr₃, -OCH₂CH₂F, -OCH₂CHF₂, and -OCH₂CF₃.

C₁₋₇hydroxyalkyl: The term "C₁₋₇hydroxyalkyl group," as used herein, pertains to a C₁₋₇alkyl group in which at least one hydrogen atom has been replaced with a hydroxy group. Examples of C₁₋₇hydroxyalkyl groups include, but are not limited
20 to, -CH₂OH, -CH₂CH₂OH, and -CH(OH)CH₂OH.

C₁₋₇carboxyalkyl: The term "C₁₋₇carboxyalkyl group," as used herein, pertains to a C₁₋₇alkyl group in which at least one hydrogen atom has been replaced with a carboxy group. Examples of C₁₋₇carboxyalkyl groups include, but are not limited
25 to, -CH₂COOH and -CH₂CH₂COOH.

C₁₋₇aminoalkyl: The term "C₁₋₇aminoalkyl group," as used herein, pertains to a C₁₋₇alkyl group in which at least one hydrogen atom has been replaced with an amino group. Examples of C₁₋₇aminoalkyl groups include, but are not limited to,
30 -CH₂NH₂, -CH₂CH₂NH₂, and -CH₂CH₂N(CH₃)₂.

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C₁₋₇alkyl-C₅₋₂₀aryl: The term "C₁₋₇alkyl-C₅₋₂₀aryl," as used herein, describes certain C₅₋₂₀aryl groups which have been substituted with a C₁₋₇alkyl group. Examples of such groups include, but are not limited to, tolyl (as in toluene), xylyl (as in xylene), mesityl (as in mesitylene), styryl (as in styrene), and cumenyl (as in cumene).

C₁₋₇alkyl-C₅₋₂₀aryloxy: The term "C₁₋₇alkyl-C₅₋₂₀aryloxy," as used herein, describes certain C₅₋₂₀aryloxy groups which have been substituted with a C₁₋₇alkyl group. Examples of such groups include, but are not limited to, tolyloxy, xylyloxy, mesityloxy, and cumenyloxy.

C₅₋₂₀aryl-C₁₋₇alkyl: The term "C₅₋₂₀aryl-C₁₋₇alkyl," as used herein, describes certain C₁₋₇alkyl groups which have been substituted with a C₅₋₂₀aryl group. Examples of such groups include, but are not limited to, benzyl (phenylmethyl), tolylmethyl, phenylethyl, and triphenylmethyl (trityl).

C₅₋₂₀aryl-C₁₋₇alkoxy: The term "C₅₋₂₀aryl-C₁₋₇alkoxy," as used herein, describes certain C₁₋₇alkoxy groups which have been substituted with a C₅₋₂₀aryl group. Examples of such groups include, but are not limited to, benzyloxy, tolylmethoxy, and phenylethoxy.

C₅₋₂₀haloaryl: The term "C₅₋₂₀haloaryl," as used herein, describes certain C₅₋₂₀aryl groups which have been substituted with one or more halo groups. Examples of such groups include, but are not limited to, halophenyl (e.g., fluorophenyl, chlorophenyl, bromophenyl, or iodophenyl, whether ortho-, meta-, or para-substituted), dihalophenyl, trihalophenyl, tetrahalophenyl, and pentahalophenyl.

Isomers, Salts, Solvates, and Protected Forms

Certain compounds may exist in one or more particular geometric, optical, enantiomeric, diastereomeric, epimeric, stereoisomeric, tautomeric, conformational, or anomeric forms, including but not limited to, cis- and trans-

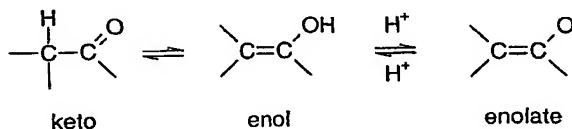
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forms; E- and Z-forms; c-, t-, and r- forms; endo- and exo-forms; R-, S-, and meso-forms; D- and L-forms; d- and l-forms; (+) and (-) forms; keto-, enol-, and enolate-forms; syn- and anti-forms; synclinal- and anticlinal-forms; α - and β - forms; axial and equatorial forms; boat-, chair-, twist-, envelope-, and halfchair- forms; and combinations thereof, hereinafter collectively referred to as "isomers" (or "isomeric forms").

Note that, except as discussed below for tautomeric forms, specifically excluded from the term "isomers," as used herein, are structural (or constitutional) isomers (i.e., isomers which differ in the connections between atoms rather than merely by the position of atoms in space). For example, a reference to a methoxy group, -OCH₃, is not to be construed as a reference to its structural isomer, a hydroxymethyl group, -CH₂OH. Similarly, a reference to ortho-chlorophenyl is not to be construed as a reference to its structural isomer, meta-chlorophenyl.

However, a reference to a class of structures may well include structurally isomeric forms falling within that class (e.g., C₁₋₇alkyl includes n-propyl and isopropyl; butyl includes n-, iso-, sec-, and tert-butyl; methoxyphenyl includes ortho-, meta-, and para-methoxyphenyl).

The above exclusion does not pertain to tautomeric forms, for example, keto-, enol-, and enolate-forms, as in, for example, the following tautomeric pairs: keto/enol (illustrated below), imine/enamine, amide/imino alcohol, amidine/amidine, nitroso/oxime, thioketone/enethiol, N-nitroso/hydroxyazo, and nitro/aci-nitro.



25

Note that specifically included in the term "isomer" are compounds with one or more isotopic substitutions. For example, H may be in any isotopic form, including ¹H, ²H (D), and ³H (T); C may be in any isotopic form, including ¹²C, ¹³C, and ¹⁴C; O may be in any isotopic form, including ¹⁶O and ¹⁸O; and the like.

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Unless otherwise specified, a reference to a particular compound includes all such isomeric forms, including (wholly or partially) racemic and other mixtures thereof. Methods for the preparation (e.g., asymmetric synthesis) and separation
5 (e.g., fractional crystallisation and chromatographic means) of such isomeric forms are either known in the art or are readily obtained by adapting the methods taught herein, or known methods, in a known manner.

Unless otherwise specified, a reference to a particular compound also includes
10 ionic, salt, solvate, and protected forms of thereof.

Solvents

Solvents may conveniently be classified according to one or more of their
15 physical or chemical properties.

For example, solvents may be classified according to their polarity, that is, their permanent dipole moment. Examples of highly polar solvents include dimethylformamide (DMF), dimethylacetamide, and acetonitrile (ACN). Examples
20 of moderately polar solvents include acetone, methanol, tetrahydrofuran (THF), ethyl acetate (AcOEt), and water. Examples of relatively non-polar solvents include diethyl ether, chloroform, and dichloromethane (DCM). Examples of non-polar and virtually non-polar solvents include alkanes, benzene, toluene, and carbon tetrachloride.

25 Solvents may also be classified as "protic" or "aprotic" according to their proton-exchange properties. Protic solvents accept and/or donate protons. Examples of protic solvents include water, alcohols, carboxylic acids (e.g., acetic acid), and amines (e.g., ammonia, pyridine). Aprotic solvents neither accept nor donate
30 protons. Examples of aprotic solvents include carbon tetrachloride, chloroform, dichloromethane (DCM), acetonitrile (ACN), ethyl acetate (AcOEt), dimethylacetamide, tetrahydrofuran (THF), dimethylformamide (DMF), toluene,

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benzene, acetone, ethers (e.g., diethyl ether), alkanes (e.g., hexane), dimethylsulfoxide (DMSO), sulfur dioxide, hexamethylphosphoramide (HMPA), and, tetramethylurea. Amphoteric solvents, such as water, are capable of both accepting and donating protons.

5

Solvents may also be classified as "organic" or "inorganic" according to their chemical composition. Conventionally, organic solvents comprise, at least, carbon atoms, while inorganic solvents do not. Examples of inorganic solvents include water, ammonia, and sulfur dioxide.

10

Advantages of the Present Invention

Advantageously, the methods of the present invention permit an improved overall yield of prodrug, **11**, **12** or **13**, calculated with respect to glutamic acid, **14**, which is much greater than that obtained using previously published methods (see, for example, Heaton, D. W., et al., 1996). The present method gives a yield, for **12A**, of 55.4% with respect to glutamic acid, **14**, whereas the previously published method gives a yield of only 13.2%.

20 Hydroxyethylation of benzyloxyaniline, **1A**, with ethylene oxide, gives the diol, **2A**, in 84% yield. Protection of the diol, **2A**, with silyl chloride and imidazole in DMF, gives the benzyl ether silyloxy compound, **3A**, in 95% yield, after purification. Deprotection of the benzyl ether silyloxy compound, **3A**, by catalytic hydrogenation, gives the silyloxy phenol, **4A**, in 100% yield. Thus, the silyloxy phenol, **4A**, is obtained in (84%)(95%)(100%) or 79.8% yield with respect to the benzyloxyaniline, **1A**.

Di-t-butyl glutamate, **15A**, was prepared by reaction of glutamic acid, **14**, with isobutylene in the presence of sulfuric acid, in 83% yield, a better yield than that reported in the literature (see, for example, Ferenz, C.R., et al., 1989).

30

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4-[N,N-bis(2-(t-butyldimethylsilyloxy)ethyl) amino]-phenol, **4A**, is converted to chloroformate, **5A**, and chloroformate, **5A**, is coupled with di-t-butyl glutamate, **15A**, to afford **6A**, in a total yield of 90% with respect to glutamate, **15A**.

- 5 The bis-silyl ether, **6A**, is cleanly deprotected with $\text{NEt}_3 \cdot 3\text{HF}$ to give the key intermediate, **7A**, in 97% yield. After a simple work up (extraction with water and Na_2CO_3), **7A** is obtained without need of further purification.

- 10 Mesylation of the bis hydroxy compound, **7A**, with mesyl anhydride in dichloromethane, gives the bis-mesyl compound, **8A**. After aqueous extraction with citric acid, the bis-mesyl compound, **8A**, is converted to the bis-iodo compound, **9A**, by treatment with NaI in refluxing acetone, in 85% yield after purification, with respect to **7A**.

- 15 Final deprotection of the bis-iodo compound, **9A**, with TFA and recrystallisation from toluene:ethyl acetate, yielded the prodrug, **12A**, in 90% yield.

- The overall yield of prodrug, **12A**, with respect to glutamic acid, **14**, is (83%)(90%)(97%)(85%)(90%) or 55.4%, more than three times higher than the
20 13.2% yield of the previously published method (see for example, Heaton, D. W., et al., 1996).

- Also advantageously, in the methods of the present invention, the synthesis of the key intermediate, **7A**, is much improved, avoiding the low yield steps of:
25 (a) 4-nitrophenyl chloroformate coupling (77% with respect to glutamic acid), (b) reduction of nitroderivative to amine (58%); and, (c) hydroxyethylation, which is generally a difficult and low yield reaction (49%).

- Also advantageously, in the methods of the present invention, the coupling of **5A**
30 with **15A** is reliable and proceeds with higher yield than the coupling of 4-nitrophenyl chloroformate with glutamic acid, which often produces ureas as by-products.

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Also advantageously, in the methods of the present invention, (a) hydroxyethylation is performed on 4-benzyloxyaniline, **1A**, instead of the more expensive and advanced intermediate di-t-butyl, N-(4-amino-phenoxy-carbonyl)-L-glutamate, (b) this step proceeds with yields up to 84% and (c) the work-up is simplified.

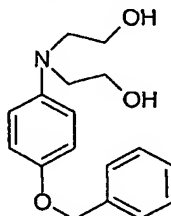
Also advantageously, in the methods of the present invention, the glutamate is introduced at a later step, and so the overall process is more economic in this expensive starting material.

EXAMPLES

The following examples are provided solely to illustrate the present invention and are not intended to limit the scope of the invention, as described herein.

Example 1

4-Benzyloxy-N,N-bis(2-hydroxyethyl)aniline (**2A**)

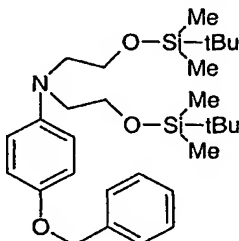


4-Benzyloxyaniline hydrochloride **1A** (9.32 g, 39.5 mmol) was dissolved in acetic acid (400 mL), triethylamine (6 mL, 43 mmol) was added followed by ethylene oxide (20 mL). The reaction mixture was stirred at room temperature for 72 h, the solvent was evaporated in a rotary evaporator under water pump then under oil pump at 50°C for 1 h. The residue was then diluted with water (500 mL) and the precipitate recovered by filtration, washed with more water, then dried in dessicator over P₂O₅ to afford **2A** (9.6 g, 84%) as a pale brown powder.

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$^1\text{H-NMR}$ (DMSO-d_6) δ_{H} : 3.32 (t, 4H, $\text{N}(\text{CH}_2\text{CH}_2\text{OH})_2$), 3.42-3.52 (m, 4H, $\text{N}(\text{CH}_2\text{CH}_2\text{OH})_2$), 4.65 (t, 2H, OH, $J=5.90$ Hz), 4.97 (s, 2H, PhCH_2), 6.60 (d, 2H, $\text{H}_{\text{arom}3+5}$, $J=9.10$ Hz), 6.83 (d, 2H, $\text{H}_{\text{arom}2+6}$), 7.25-7.44 (m, 5H, $\text{H}_{\text{arom benzyl}}$).

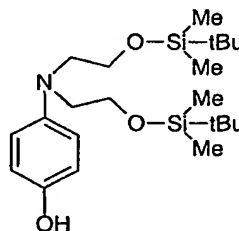
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Example 24-Benzyloxy-N,N-bis[2-(t-butyldimethylsilyloxy)ethyl]aniline (**3A**)

A mixture of **2A** (9.6 g, 33.4 mmol), t-butyldimethylsilyl chloride (12.7 g, 84 mmol) and imidazole (7.8 g, 130 mmol) were dissolved in dry DMF (80 mL) and the reaction stirred overnight, then the solvent was evaporated, the residue taken in dichloromethane, the precipitated imidazole hydrochloride filtered off, and the filtrate evaporated. The residue was purified by column chromatography on silica (cyclohexane:ethyl acetate 4:1), to afford **3A** (16.4 g, 95%) as an oil.

$^1\text{H-NMR}$ (DMSO-d_6) δ_{H} : -0.01 (s, 12H, $2\times\text{SiMe}_2$), 0.84 (s, 18H, $2\times\text{Si}t\text{-Bu}$), 3.39 (t, 4H, $\text{N}(\text{CH}_2\text{CH}_2\text{OSi})_2$, $J=5.98$ Hz), 3.67 (t, 4H, $\text{N}(\text{CH}_2\text{CH}_2\text{OSi})_2$), 4.96 (s, 2H, PhCH_2), 6.59 (d, 2H, $\text{H}_{\text{arom}3+5}$, $J=9.03$ Hz), 6.82 (d, 2H, $\text{H}_{\text{arom}2+6}$), 7.25-7.44 (m, 5H, $\text{H}_{\text{arom benzyl}}$).

20

Example 34-{N,N-bis[2-(t-butyldimethylsilyloxy)ethyl]amino}-phenol (**4A**)

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The benzyl ether **3A** (11.4 g, 22.1 mmol) was dissolved in THF, Pd/C catalyst (1.6 g) was added and the suspension stirred overnight under H₂ atmosphere. The catalyst was filtered off, the solvent evaporated to afford **4A** (9.4 g, 100%) as an oil.

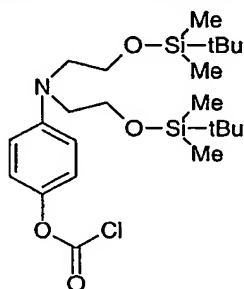
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¹H-NMR (DMSO-d₆) δ_H: 0.00 (s, 12H, SiMe₂), 0.84 (s, 18H, Sit-Bu), 3.34 (t, 4H, N(CH₂CH₂OSi)₂, J=6.07 Hz), 3.65 (t, 4H, N(CH₂CH₂OSi)₂), 6.51 (d, 2H, H_{arom2+6}, J=9.23 Hz), 6.58 (d, 2H, H_{arom3+5}), 8.46 (s, 1H, OH).

10

Example 4

4-[N,N-bis(2-silyloxyethyl)amino]phenyl chloroformate (**5A**)



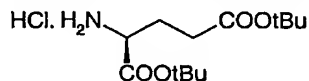
In a stirred solution of **4A** (13.2 g, 32 mmol) in toluene (120 mL), phosgene 20% solution in toluene (35 mL, 70 mmol) was added at once at room temperature.

15 After 2 min, triethylamine (5.3 mL, 38 mmol) was added dropwise, and the formation of chloroformate **5A** was complete in 10 min as detected by IR spectra ($\nu_{\text{OCCl}}=1784 \text{ cm}^{-1}$). The solution was filtered and the filtrate evaporated to afford an oil, which was dissolved in THF (80 mL) and used in the glutamate conjugation reaction.

20

Example 5

Di-t-butyl L-glutamate hydrochloride (**15A**)



25 Glutamic acid **14** (4.4 g, 30 mmol) was suspended in chloroform (120 mL) and sulfuric acid (3 mL, 56 mmol) was added. The suspension was cooled at -78°C

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and condensed isobutylene (70 mL) was added. The reaction mixture was stirred allowing to warm to room temperature for 18 h. After 18 hr, all the suspended solid had dissolved to a clear solution. Nitrogen was bubbled through the solution for 10 min, then it was extracted with aqueous NaHCO₃ (2 x 100 mL) and
5 distilled water (100 mL). The organic layer was dried (MgSO₄), evaporated, and kept in a dessicator over NaOH pellets for 48 h to afford **15** (6.7 g, 86%) as an oil.

¹H-NMR δ_H: 1.39 (s, 9H, COO-t-Bu), 1.41 (s, 9H, COO-t-Bu), 1.60-1.90 (2m, 2H, CH₂CH(NH₂)-), 2.15-2.35 (m, 2H, CH₂COO), 2.85 (s, 2H, NH₂), 3.20-3.35 (m, 1H,
10 CH(NH₂)-). MS (ESI): 260 (M⁺ + H, 100), 283 (M⁺ + Na, 10).

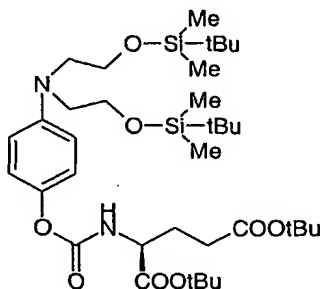
Glutamate, **15** (604 mg, 2.33 mmol) was dissolved in dry diethyl ether (50 mL) and 1 M HCl in diethyl ether (2.33 mL, 1 eq.) was added. The solution was stirred for 5 min, then the solvent was diluted with hexane, and then evaporated.
15 The residue dissolved partially in hexane without crystallisation; therefore it was redissolved in diethyl ether, and 1 M HCl in diethyl ether (2 mL, 0.86 eq.) was added. The solution was stirred for 10 min, then the solvent was diluted with hexane and heated at reflux for 5 min. The solution was cooled on ice bath at room temperature, then was evaporated to afford an oily residue. The residue
20 was scratched with hexane and was left for 18 h in hexane to crystallise. The crystals were washed with hexane, to afford **15.HCl (15A)** as a white solid (624 mg). The hexane washing afforded a second crop of crystals of **15.HCl (15A)** (38 mg). The total yield of **15.HCl (15A)** was 662 mg (96%) and the yield of esterification, hydrochloride formation, and crystallisation is (86%)(96%) = 83%.

25 ¹H-NMR δ_H: 1.40 (s, 9H, COO-t-Bu), 1.45 (s, 9H, COO-t-Bu), 1.90-2.01 (q, 2H, CH₂CH(NH₂)-), 2.20-2.50 (m, 2H, CH₂COO), 3.87 (t, 1H, CH(NH₂)-, J=6.62 Hz), 8.40 (s, 3H, NH₃⁺).

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Example 6

Di-t-butyl, N-{4-[N,N-bis(2-(t-butyl dimethylsilyloxy)ethyl) amino]-
phenoxy carbonyl}-L-glutamate (**6A**)

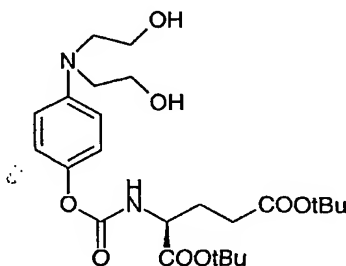


- 5 Di-t-butyl L-glutamate hydrochloride **15A** (9.77 g, 33 mmol) was dissolved in ethyl acetate (100 mL) and extracted with aq. Na_2CO_3 (100 mL). The organic layer was dried (MgSO_4) and evaporated. The oily residue was dissolved in THF (80 mL). The solution of chloroformate **5A** (obtained from 13.2 g **4A** as described in Example 4) in THF was poured over the solution of di-t-butyl glutamate **15A**
- 10 under stirring, at room temperature, followed by triethylamine (4.9 mL, 35 mmol). After 5 min the reaction was completed, as monitored by IR (the peak of chloroformate $\nu_{\text{COCOCl}}=1784\text{ cm}^{-1}$ disappeared). The precipitate was filtered off, the solvent evaporated and the residue purified by column chromatography on silica (cyclohexane:ethyl acetate 4:1) to afford **6A** (20.45 g, 90%) as an oil.
- 15 $^1\text{H-NMR}$ (DMSO-d_6) δ_{H} : 0.00 (s, 12H, $2\times\text{SiMe}_2$), 0.84 (s, 18H, $2\times\text{Si-t-Bu}$), 1.39 (s, 9H, COO-t-Bu), 1.40 (s, 9H, COO-t-Bu), 1.75-2.05 (2m, 2H, $\text{CH}_2\text{CH}(\text{NH})-$), 2.32 (t, 2H, CH_2COO , $J=7.69\text{ Hz}$), 3.44 (t, 4H, $\text{N}(\text{CH}_2\text{CH}_2\text{OSi})_2$, $J=5.83\text{ Hz}$), 3.70 (t, 4H, $\text{N}(\text{CH}_2\text{CH}_2\text{OSi})_2$), 3.90-4.05 (m, 1H, $\text{CH}(\text{NH})-$), 6.61 (d, 2H, $\text{H}_{\text{arom}2+6}$, $J=9.10\text{ Hz}$), 6.83 (d, 2H, $\text{H}_{\text{arom}3+5}$), 7.87 (d, 1H, NH-G , $J=7.85\text{ Hz}$).
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Example 7

Di-t-butyl, N-{4-[N,N-bis(2-hydroxyethyl)amino]-phenoxy-carbonyl}-L-glutamate
(7A)



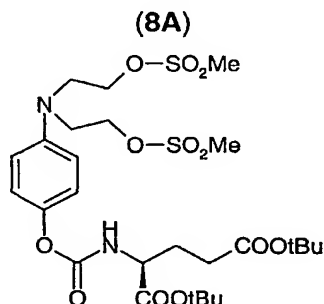
- 5 $\text{NEt}_3 \cdot 3\text{HF}$ (42 mL) was added to a solution of **6A** (20.45 g, 28.8 mmol) in THF (315 mL). The solution was stirred at room temperature for 12 h, then the solvent was evaporated, the residue was taken in ethyl acetate (100 mL) and extracted with water (100 mL), aq. Na_2CO_3 (100 mL) and water again (100 mL). The organic layer was dried (MgSO_4) and evaporated to afford **7A** (13.4 g, 97%) as a
10 foamy solid.

- $^1\text{H-NMR}$ (DMSO-d_6) δ_{H} : 1.39 (s, 9H, COO-t-Bu), 1.40 (s, 9H, COO-t-Bu), 1.70-2.00 (2m, 2H, $\text{CH}_2\text{CH}(\text{NH})-$), 2.32 (t, 2H, CH_2COO , $J=7.63$ Hz), 3.37 (t, 4H, $\text{N}(\text{CH}_2\text{CH}_2\text{OH})_2$, $J=5.74$ Hz), 3.50 (t, 4H, $\text{N}(\text{CH}_2\text{CH}_2\text{OH})_2$), 3.90-4.05 (m, 1H, $\text{CH}(\text{NH})-$), 4.71 (t, 2H, OH, $J=5.29$ Hz), 6.62 (d, 2H, $\text{H}_{\text{arom}2+6}$, $J=8.98$ Hz), 6.82 (d, 2H, $\text{H}_{\text{arom}3+5}$), 7.88 (d, 1H, NH-G, $J=7.90$ Hz).
15

Example 8

Di-t-butyl, N-{4-[N,N-bis(2-mesyloxyethyl)amino]-phenoxy-carbonyl}-L-glutamate

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The bis-hydroxy compound **7A** (1.73 g, 3.58 mmol) and DMAP (29 mg, 0.24 mmol) were dissolved in dry dichloromethane (50 mL), and cooled in an ice bath under stirring. The cooled solution was treated with a solution of methanesulfonic anhydride (2.7 g, 15.5 mmol) and triethylamine (2.7 mL, 19.3 mmol) in dry dichloromethane (50 mL), and was then allowed to warm up for 18 h. The reaction mixture was diluted with dichloromethane (100 mL) and extracted with 10% aq. citric acid (2x200 mL). The aqueous washings were back-extracted with dichloromethane (50 mL), and the organic fractions pooled, dried (MgSO₄) and evaporated to afford **8A** as a solid.

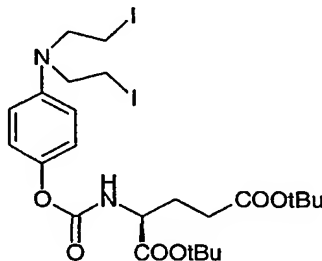
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¹H-NMR (DMSO-d₆) δ_H: 1.41 (s, 9H, COO-t-Bu), 1.42 (s, 9H, COO-t-Bu), 1.70-2.00 (2m, 2H, CH₂CH(NH)-), 2.28-2.38 (m, 2H, CH₂COO), 3.15 (s, 6H, CH₃SO₃), 3.71 (t, 4H, N(CH₂CH₂OMes)₂, J=5.69 Hz), 3.90-4.05 (m, 1H, CH(NH)-), 4.30 (t, 4H, N(CH₂CH₂OMes)₂), 6.78 (d, 2H, H_{arom2+6}, J=9.12 Hz), 6.92 (d, 2H, H_{arom3+5}), 7.92 (d, 1H, NH-G, J=7.89 Hz).

15

Example 9

Di-t-butyl, N-{4-[N,N-bis(2-iodoethyl)amino]-phenoxy-carbonyl}-L-glutamate (**9A**)



The crude product **8A** was dissolved in acetone (100 mL) and treated with NaI (7.1 g, 47.4 mmol). The reaction mixture was refluxed for 4.5 h, then filtered and evaporated. The residue was partitioned between water (200 mL) and dichloromethane (200 mL). The organic layer was washed again with water (2x100 mL). The aqueous layers were combined and back-extracted with dichloromethane (100 mL). The organic fractions were pooled, dried (MgSO₄) and evaporated. The residue was purified by column chromatography on silica

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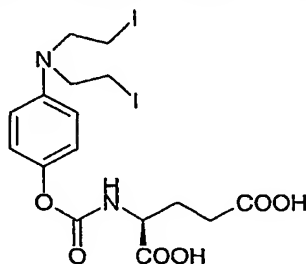
(starting with hexane: dichloromethane 1:1, then neat dichloromethane) to afford **9A** (2.13 g, overall yield 85%) as an orange solid.

¹H-NMR (DMSO-d₆) δ_H: 1.41 (s, 9H, COO-t-Bu), 1.42 (s, 9H, COO-t-Bu), 1.70-2.05 (2m, 2H, CH₂CH(NH)-), 2.29-2.39 (m, 2H, CH₂COO), 3.31 (t, 4H, N(CH₂CH₂I)₂, J=7.70 Hz), 3.72 (t, 4H, N(CH₂CH₂I)₂), 3.85-4.00 (m, 1H, CH(NH)-), 6.67 (d, 2H, H_{arom2+6}, J=9.01 Hz), 6.93 (d, 2H, H_{arom3+5}), 7.97 (d, 1H, NH-G, J=7.83 Hz).

10

Example 10

N-{4-[N,N-bis(2-iodoethyl)amino]-phenoxy-carbonyl}-L-glutamic acid (**12A**)



The di-t-butyl ester **9A** (1.7 g, 2.42 mmol) was dissolved in TFA (45 mL) and stirred for 55 min at room temperature. The reaction mixture was evaporated 5 times after addition of ethyl acetate (under nitrogen), redissolved in ethyl acetate (5 mL) and toluene (50 mL) was added. The solution was seeded with crystals of **12A**. The solution was stored at -20°C overnight. The formed crystals were recovered by filtration, washed with toluene:ethyl acetate 10:1 and dried to afford **12A** (180 mg). The filtrate was concentrated in a rotary evaporator until the first crystals starts to appear, then it was stored overnight at -20 °C. A second crop of crystals of **12A** (880 mg) was obtained. Repeating the steps described above a third crop (230 mg) was obtained. All 3 crops of crystals were pure by ¹H-NMR. A total of 1.29 g (90%) of **12A** was obtained.

¹H-NMR (DMSO-d₆) δ_H: 1.75-2.10 (2m, 2H, CH₂CH(NH)-), 2.36 (t, 2H, CH₂COO, J=8.15 Hz), 3.31 (t, 4H, N(CH₂CH₂I)₂, J=7.87 Hz), 3.71 (t, 4H, N(CH₂CH₂I)₂),

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3.95-4.05 (m, 1H, CH(NH)-), 6.65 (d, 2H, H_{arom2+6}, J=9.08 Hz), 6.94 (d, 2H, H_{arom3+5}), 7.94 (d, 1H, NH-G, J=8.10 Hz).

REFERENCES

5

A number of patents and publications are cited above in order to more fully describe and disclose the invention and the state of the art to which the invention pertains. Full citations for these references are provided below. Each of these references is incorporated herein by reference in its entirety into the present disclosure.

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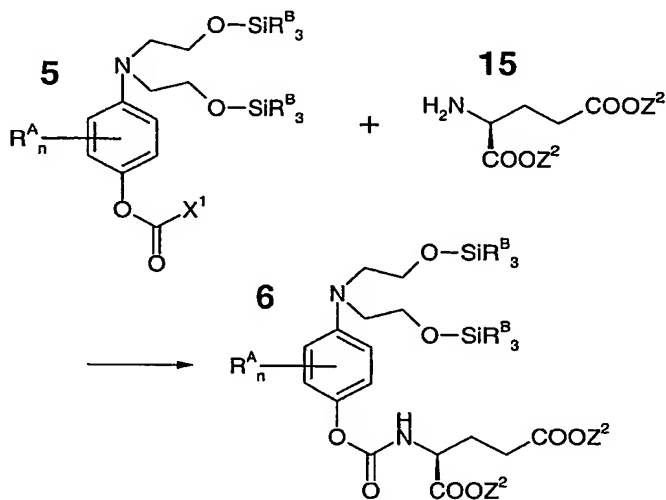
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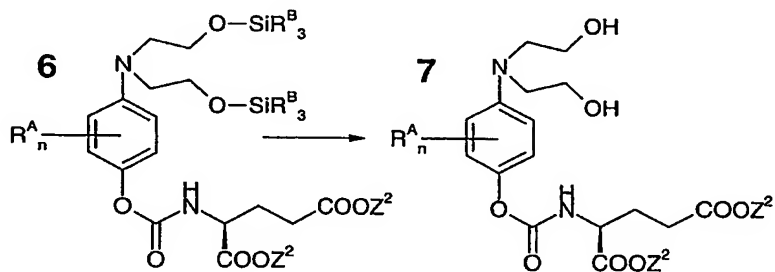
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CLAIMS

1. A method of synthesis comprising the steps of, in order:
 glutamate conjugation (GC), in which a
 5 4-[N,N-bis(2-silyloxyethyl)amino]phenyl haloformate, activated carbonate,
 or activated carbamate **5**, reacts with a glutamate, **15**, to give an
 N-{4-[N,N-bis(2-silyloxyethyl)amino] phenoxy-carbonyl}-L-glutamate, **6**:

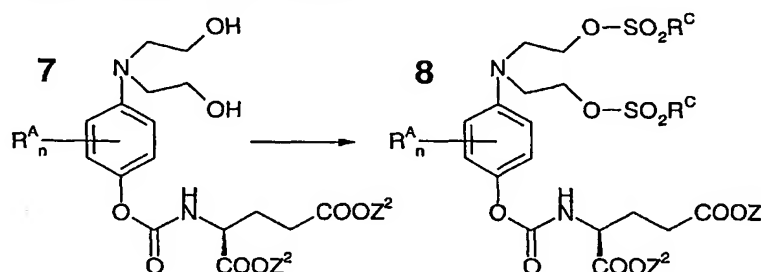


- silyloxy deprotection (SD), in which said
 10 N-{4-[N,N-bis(2-silyloxyethyl)amino]phenoxy-carbonyl}-L-glutamate, **6**, is
 converted to an N-{4-[N,N-bis(2-hydroxyethyl)amino]phenoxy-carbonyl}-
 L-glutamate, **7**:



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sulfonic esterification (SE), in which said N-{4-[N,N-bis(2-hydroxyethyl)amino]phenoxy carbonyl}-L-glutamate, **7**, is converted to an N-{4-[N,N-bis(2-sulfonyloxyethyl)amino]phenoxy carbonyl}-L-glutamate, **8**:



wherein:

X^1 is a leaving group;

n is an integer from 0 to 4;

each R^A is independently an aryl substituent;

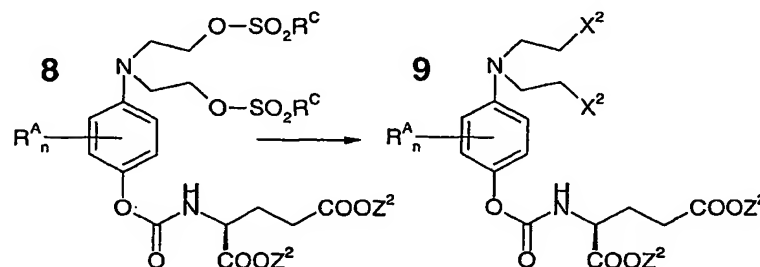
each R^B is a silyl substituent, and is independently C_{1-7} alkyl, C_{3-20} heterocyclyl, or C_{5-20} aryl, and is optionally substituted;

each R^C is a sulfonic substituent, and is independently C_{1-7} alkyl, C_{3-20} heterocyclyl, or C_{5-20} aryl, and is optionally substituted; and,

each Z^2 is independently a carboxylic acid protecting group.

2. A method according to claim 1, further comprising, following the step of sulfonic esterification (SE), the additional step of:

halogenation (HL), in which said N-{4-[N,N-bis(2-sulfonyloxyethyl)amino]phenoxy carbonyl}-L-glutamate, **8**, is converted to an N-{4-[N,N-bis(2-haloethyl)amino]phenoxy carbonyl}-L-glutamate, **9**:

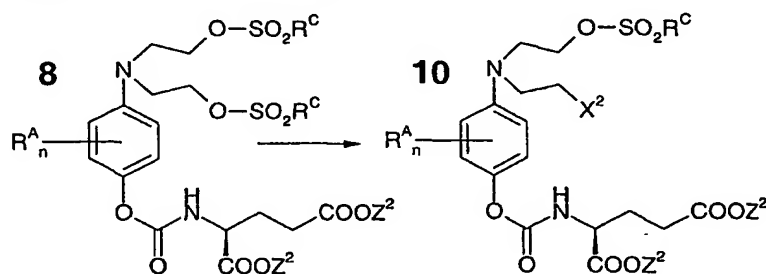


wherein each X^2 is independently -F, -Cl, -Br, -I.

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3. A method according to claim 1, further comprising, following the step of sulfonic esterification (SE), the additional step of:

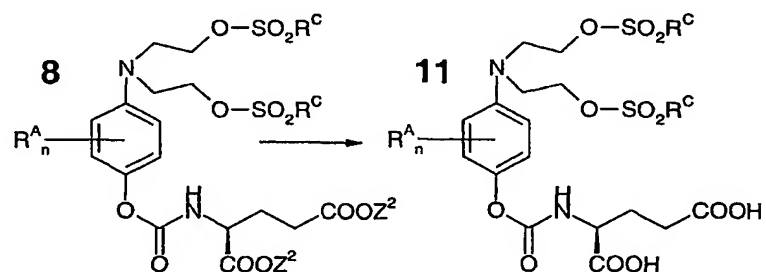
5 halogenation (HL), in which said N-{4-[N,N-bis(2-sulfonyloxyethyl)amino]phenoxy carbonyl}-L-glutamate, **8**, is converted to an N-{4-[N-(2-sulfonyloxy ethyl),N-(2-haloethyl)amino]phenoxy carbonyl}-L-glutamate, **10**:



wherein each X^2 is independently -F, -Cl, -Br, -I.

- 10 4. A method according to claim 1, further comprising, following the step of sulfonic esterification (SE), the additional step of:

glutamate deprotection (GD), in which said N-{4-[N,N-bis(2-sulfonyloxyethyl)amino]phenoxy carbonyl}-L-glutamate, **8**, is converted to an N-{4-[N,N-bis(2-sulfonyloxyethyl)amino]phenoxy carbonyl}-L-glutamic acid, **11**:



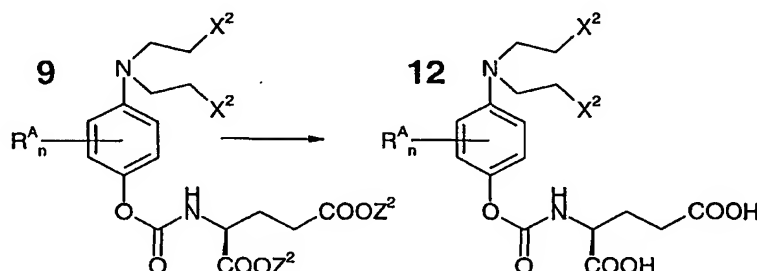
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5. A method according to claim 2, further comprising, following the step of halogenation (HL), the additional step of:

glutamate deprotection (GD), in which said

N-{4-[N,N-bis(2-haloethyl)amino] phenoxy carbonyl}-L-glutamate, **9**, is

- 5 converted to an N-{4-[N,N-bis (2-haloethyl)amino]phenoxy carbonyl}-L-glutamic acid, **12**:

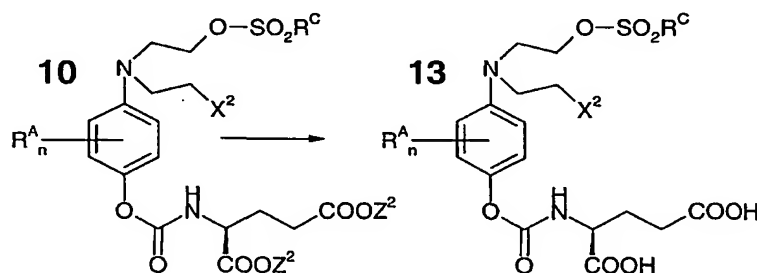


6. A method according to claim 3, further comprising, following the step of halogenation (HL), the additional step of:

glutamate deprotection (GD), in which said N-{4-[N-(2-sulfonyloxy

ethyl),N-(2-haloethyl)amino] phenoxy carbonyl}-L-glutamate, **10**, is

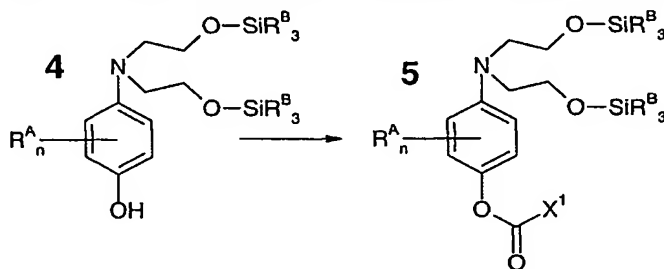
converted to an N-{4-[N-(2-sulfonyloxyethyl),N-(2-haloethyl)amino] phenoxy carbonyl}-L-glutamic acid, **13**:



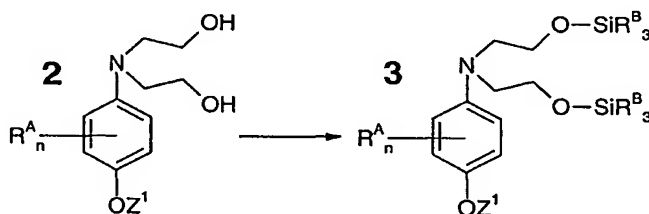
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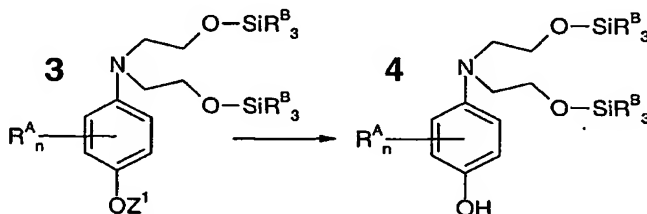
7. A method according to any one of claims 1 to 6, further comprising, preceding the step of glutamate conjugation (GC), the additional step of: activation (AC), in which a 4-[N,N-bis(2-silyloxyethyl)amino]phenol, **4**, is converted to said 4-[N,N-bis(2-silyloxyethyl)amino]phenyl haloformate, activated carbonate, or activated carbamate **5**:



8. A method according to claim 7, further comprising, preceding the step of activation (AC), the additional steps of: silyloxy protection (SP), in which a 4-(protected hydroxy)-N,N-bis(2-hydroxyethyl)aniline, **2**, is converted to a 4-(protected hydroxy)-N,N-bis(2-silyloxyethyl) aniline, **3**:



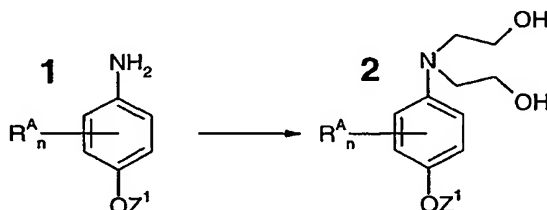
- phenolic deprotection (PD), in which said 4-(protected hydroxy)-N,N-bis(2-silyloxyethyl)aniline, **3**, is converted to said 4-[N,N-bis(2-silyloxyethyl)amino] phenol, **4**:



wherein Z^1 is a phenol protecting group.

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9. A method according to claim 8, further comprising, preceding the step of silyloxy protection (SP), the additional step of:
 amine substitution (AS), in which a 4-(protected hydroxy)-aniline, **1**,
 is converted to said 4-(protected hydroxy)-N,N-bis(2-hydroxyethyl)aniline,
2:



10. A method according to any one of claims 1 to 9, further comprising, at any time preceding the step of glutamate conjugation (GC), the parallel step of:
 glutamate protection (GP), in which L-glutamic acid, **14**, is converted to said L-glutamate, **15**:



11. A method according to any one of claims 1 to 10, wherein said glutamate conjugation (GC) step is achieved by reaction in the presence of a base.
12. A method according to any one of claims 1 to 10, wherein said glutamate conjugation (GC) step is achieved by reaction in the presence of the base triethylamine.
13. A method according to any one of claims 1 to 12, wherein said silyloxy deprotection (SD) step is achieved by reaction with triethylamine trihydrofluoride ($\text{NEt}_3 \cdot 3\text{HF}$).

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14. A method according to any one of claims 1 to 13, wherein said sulfonic esterification (SE) step is achieved by reaction with a sulfonic anhydride, $(R^C SO_2)_2O$.
- 5 15. A method according to any one of claims 1 to 13, wherein said sulfonic esterification (SE) step is achieved by reaction with a sulfonic anhydride, $(R^C SO_2)_2O$, in the presence of a base.
- 10 16. A method according to any one of claims 1 to 13, wherein said sulfonic esterification (SE) step is achieved by reaction with a sulfonic anhydride, $(R^C SO_2)_2O$, in the presence of one or both of triethylamine and 4-dimethylaminopyridine.
- 15 17. A method according to any one of claims 14 to 16, wherein said sulfonic anhydride is methanesulfonic anhydride, $(MeSO_2)_2O$.
18. A method according to any one of claims 2, 3, or 5 to 17, wherein said halogenation (HL) step is achieved by reaction with an alkaline halide.
- 20 19. A method according to claim 18, wherein said alkaline halide is sodium iodide.
20. A method according to any one of claims 4 to 19, wherein said glutamate deprotection (GD) step is achieved by reaction with acid.
- 25 21. A method according to any one of claims 4 to 19, wherein said glutamate deprotection (GD) step is achieved by reaction with trifluoroacetic acid.
- 30 22. A method according to any one of claims 7 to 21, wherein said activation (AC) step is achieved by reaction with a carbonyl halide, $X^1C(=O)X^1$.

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23. A method according to any one of claims 7 to 21, wherein said activation (AC) step is achieved by reaction with a carbonyl halide, $X^1C(=O)X^1$ in the presence of a base.
- 5 24. A method according to any one of claims 7 to 21, wherein said activation (AC) step is achieved by reaction with a carbonyl halide, $X^1C(=O)X^1$ in the presence of the base triethylamine.
- 10 25. A method according to any one of claims 22 to 24, wherein said carbonyl halide is phosgene, $ClC(=O)Cl$.
26. A method according to any one of claims 8 to 25, wherein said silyloxy protection (SP) step is achieved by reaction with the corresponding halosilane, SiR^B_3X .
- 15 27. A method according to any one of claims 8 to 25, wherein said silyloxy protection (SP) step is achieved by reaction with the corresponding chlorosilane, SiR^B_3Cl .
- 20 28. A method according to any one of claims 8 to 25, wherein said silyloxy protection (SP) step is achieved by reaction with the corresponding halosilane, SiR^B_3X , in the presence of a base.
- 25 29. A method according to any one of claims 8 to 25, wherein said silyloxy protection (SP) step is achieved by reaction with the corresponding halosilane, SiR^B_3X , in the presence of the base imidazole.
- 30 30. A method according to any one of claims 8 to 29, wherein said phenolic deprotection (PD) step is achieved by treatment with hydrogen gas and a palladium/carbon catalyst.

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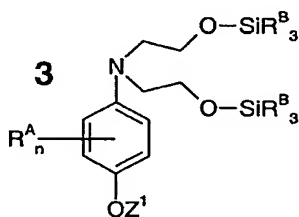
31. A method according to any one of claims 9 to 30, wherein said amine substitution (AS) step is achieved by treatment with ethylene oxide.
- 5 32. A method according to any one of claims 9 to 30, wherein said amine substitution (AS) step is achieved by treatment with ethylene oxide in acetic acid, in the presence of a base.
- 10 33. A method according to any one of claims 9 to 30, wherein said amine substitution (AS) step is achieved by treatment with ethylene oxide in acetic acid, in the presence of the base triethylamine.
- 15 34. A method according to any one of claims 10 to 33, wherein said glutamate protection (GP) step is achieved by reaction with isobutylene in the presence of sulfuric acid.
35. A method according to any one of claims 1 to 34, wherein X^1 is -F, -Cl, -Br, p-nitrophenoxy, pentafluorophenoxy, succinimidyloxy, or imidazolyl.
- 20 36. A method according to any one of claims 1 to 34, wherein X^1 is -F, -Cl, or -Br.
37. A method according to any one of claims 1 to 34, wherein X^1 is -Cl.
- 25 38. A method according to any one of claims 1 to 34, wherein X^1 is p-nitrophenoxy, pentafluorophenoxy, succinimidyloxy.
39. A method according to any one of claims 1 to 34, wherein X^1 is imidazolyl.
- 30 40. A method according to any one of claims 1 to 39, wherein each of R^{A2} , R^{A3} , R^{A5} , and R^{A6} is independently -H or an aryl substituent selected from: halo; ether; thioether; acyl; ester; amido; disubstituted amino; cyano; nitro; and, C_{1-7} alkyl.

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41. A method according to any one of claims 1 to 39, wherein n is 0 or each R^A is -H.
- 5 42. A method according to any one of claims 1 to 41, wherein each R^B is independently -Me, -Et, -nPr, -iPr, -nBu, -sBu, -iBu, -tBu, or -Ph.
43. A method according to any one of claims 1 to 41, wherein each $-SiR^B_3$ group is independently $-Si(Me)_3$, $-Si(Et)_3$, $-Si(iPr)_3$, $-Si(tBu)(Me)_2$, or
10 $-Si(Ph)_2(tBu)$.
44. A method according to any one of claims 1 to 41, wherein each $-SiR^B_3$ groups is $-Si(tBu)(Me)_2$.
- 15 45. A method according to any one of claims 1 to 44, wherein each R^C is C_{1-7} alkyl or C_{5-20} aryl, and is optionally substituted.
46. A method according to any one of claims 1 to 44, wherein each R^C is -Me.
- 20 47. A method according to any one of claims 1 to 44, wherein each R^C is phenyl or substituted phenyl.
48. A method according to any one of claims 1 to 47, wherein each Z^2 is independently t-butyl, allyl, or benzyl.
- 25 49. A method according to any one of claims 1 to 47, wherein each Z^2 is t-butyl.
50. A method according to any one of claims 2 to 49, wherein X^2 is -Cl, -Br,
30 or -I.
51. A method according to any one of claims 2 to 49, wherein X^2 is -Cl.

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52. A method according to any one of claims 2 to 49, wherein X^2 is -I.
53. A method according to any one of claims 8 to 52, wherein Z^1 is benzyl or allyl.
54. A method according to any one of claims 8 to 52, wherein Z^1 is benzyl.
55. A compound of the formula **11**, **12**, or **13** obtainable by a method comprising a method according to any one of claims 1 to 54.
56. A compound of the formula **11**, **12**, or **13** obtained by a method comprising a method according to any one of claims 1 to 54.
57. A compound of the formula:



wherein:

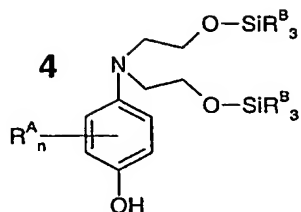
n is an integer from 0 to 4;

each R^{A} is independently an aryl substituent;

each R^{B} is a silyl substituent, and is independently C_{1-7} alkyl, C_{3-20} heterocyclyl, or C_{5-20} aryl, and is optionally substituted; and, Z^1 is a phenol protecting group.

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58. A compound of the formula:

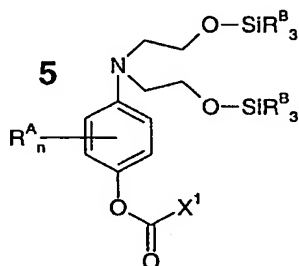


wherein:

n is an integer from 0 to 4;

5 each R^A is independently an aryl substituent; and,
each R^B is a silyl substituent, and is independently C_{1-7} alkyl,
 C_{3-20} heterocyclyl, or C_{5-20} aryl, and is optionally substituted.

59. A compound of the formula:



wherein:

X^1 is a leaving group;

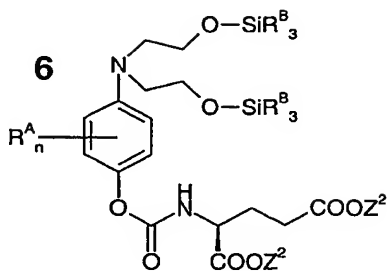
n is an integer from 0 to 4;

each R^A is independently an aryl substituent; and,

15 each R^B is a silyl substituent, and is independently C_{1-7} alkyl,
 C_{3-20} heterocyclyl, or C_{5-20} aryl, and is optionally substituted.

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60. A compound of the formula:



wherein:

n is an integer from 0 to 4;

each R^A is independently an aryl substituent;

each R^B is a silyl substituent, and is independently C_{1-7} alkyl,

C_{3-20} heterocyclyl, or C_{5-20} aryl, and is optionally substituted; and,

each Z^2 is independently a carboxylic acid protecting group.

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International Application No.

PCT/GB 02/00281

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07C269/04 C07C271/54 C07C309/66 C07F7/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07C C07F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 02/00281

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Information on patent family members

International Application No

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